DOCTORAL THESIS

Links between hydrology, community ecology and ecosystem services in the hyporheic zone of streams and rivers
an interdisciplinary perspective

Peralta-Maraver, Ignacio

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Links between hydrology, community ecology and ecosystem services in the hyporheic zone of streams and rivers: an interdisciplinary perspective.

By Ignacio Peralta-Maraver

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University of Roehampton London

hypo TRAIN

While I'm off chasing my own dreams
Sailing around the world
Please know that I'm yours to keep
My beautiful girl

"The girl" – City and Colour

Thesis abstract

1 | Streambed sediments are an interface between the surface water and the groundwater systems characterised by the simultaneous occurrence of multiple physical, biological and chemical processes. Transformation and biodegradation of nutrients and contaminants are among the important ecological services the streambed provides. Streambeds are also the biotope of a diverse and productive biota. This system is divided into the benthic zone (BZ), which is located in the upper centimetres of the streambed and the hyporheic zone (HZ), which encompasses the volume of sediment beneath the BZ where surface water interacts with groundwater. The nature of the biota and the ecosystem processes differ depending on the streambed compartment.

2 | I first reviewed how depth gradient and hydrological flux conditions shape the structure of the hyporheic assemblage (hyporheos). Metadata analysis predicted a reduction in net biomass and productivity through depth in the streambed. However, my models were based on data derived from the literature, which focussed on macroinvertebrates only, and it is likely that model predictability will increase by including Protozoa and meiofauna. Assessing the organization of the whole hyporheos is also important to better understand the self-purifying capacity of the HZ as it removes nutrients and emerging organic contaminants (EOCs) from the medium (hyporheic bioreactor ability). Based on this premise, I conceptualize how the residence time of water in pore sediments (resulting from hyporheic exchange flow) and the rest of the hyporheos might drive the hyporheic bioreactor efficiency.

3 | Then, I carried out a local-scale survey study in which I combined sampling techniques from hydrology, biochemical engineering and community ecology to determine accurately hydrological conditions and to characterise the resident assemblages at a cm resolution in the streambed. My findings showed for the first time that decline in biomass and secondary production of the different taxa is body-size dependent. Smaller organisms (i.e. protozoa) penetrate deeper and colonise more compacted and anoxic sediment layers. My results also evidenced that down-welling (DW) sites are hot spots of productivity, and therefore carbon processing in freshwater systems. I also demonstrated that hyporheos and benthic assemblages (benthos) are two measurable ecological communities with individual integrity, whose demarcation boundary reached deeper under DW conditions.

4 | Subsequently, I analysed in a microcosms experiment how dissolved organic carbon (glucose), cell density of colloidal bacteria degraders and predator–prey interactions drive the

capacity of hyporheic sediments to process a model EOCs (Ibuprofen). Glucose and the presence of the predator at medium density levels significantly promoted the degraders population growth. The increase in degraders cell density resulted in a higher consumption of Ibuprofen. Furthermore, the positive effect of predator presence interacted synergistically both with glucose availability and degrader cell density, producing an intensification of degrader population growth and Ibuprofen removal, respectively. These findings evidenced the importance of preserving the water interchange between the open channel and the HZ (and consequently the nutrient loading) and the natural predator–prey dynamics in order to promote ecosystem services upon which human well–being depends.

5 | Finally, I conducted a regional–scale study across 30 different rivers in order to further investigate leaf litter processing in the streambed. I measured different leaf litter breakdown rates by combining the cotton–strips assay and the tea bag index. Then, I modelled the obtained breakdown rates as a response of the streambed compartment (BZ and HZ) and the biological features of the streambed assemblage by involving a large range of organisms (Prokaryota, Protozoa and Eumetazoa invertebrates). Results from my models predicted a higher leaf litter breakdown in the BZ than in the HZ. Furthermore, the biomass of all the studied groups, α–diversity of Eumetazoa invertebrates and functional diversity of Prokaryota were important predictors that were positively related with the decay rates. The inferential models I presented are a suitable tool to predict the efficiency of streambed systems in the processing of leaf litter, based on the biological features of the assemblage and the difference between the BZ and the HZ.

6 | My findings covered significant knowledge-gaps on the structure of benthos and hyporheos, the mechanistic understanding of the processes and services that occur in the HZ, and their relative importance for the whole ecosystem functioning compared with those that take place in the BZ. Since the HZ was first defined the existence of a measurable transition between benthos and hyporheos had never been delimited before. Therefore, from my findings it is now possible to determine the contribution of benthos and hyporheos to the ecosystem processes and services. My research also enhanced our understanding of the processing of EOCs and alochtonous coarse organic carbon (leaf litter) in the streambed. Here I emphasise that the bioreactor ability of the streambed is sustained and maintained by diverse and active assemblages and that all size categories play an important role in its functioning.

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List of abbreviations by alphabetic order

AIC: Akaike information criterion
ANOSIM: Analysis of similarities
ANOVA: Analysis of variance
AWCD: Average well colour development
C: Carbon
CI: Confidence interval
CrI: Credible interval
BZ: Benthic zone
DOC: Dissolved organic carbon
DW: Down-welling
EOC: Emerging organic contaminant
GLMM: Generalised linear mixed model
H´: Shannon-Wienner´s diversity index
H-RS: High river stage
HZ: Hyporheic zone
K: Global litter decay coefficient
k_{cotton}: Exponential decay coefficient of cotton strips
k_{green}: Exponential decay coefficient of green-tea litter
k_{red}: Exponential decay coefficient of red-tea litter
LC-MS/MS/MS: Liquid chromatography-targeted tandem mass spectrometry
LMM: Linear mixed model
L-RS: Low river stage
OD: Optical density
S: Leaf litter stabilisation factor
SD: Standard deviation
SPE: Solid phase extraction
SSD: Sediment size distribution
TBI: Tea bag index
TED: Thermal extinction depth
T. Lance: Thermal lance
UW: Up-welling
VF: Vertical flux
WAIC: Widely applicable information criterion
WWTP: Waste water treatment plant

Contributions on data-chapters

Chapter 2 | Interplay of hydrology, community ecology and pollutant attenuation in the hyporheic zone [Ignacio Peralta–Maraver, Julia Reiss & Anne L. Robertson]: IP-M conceived this review and lead the preparation of the manuscript. IP-M carried out the statistical analysis of metadata and interpretation of the results. IP-M wrote the manuscript, with significant contributions from JR and ALR

Chapter 3 | Environmental filtering and community delineation in the streambed ecotone [Ignacio Peralta-Maraver, Jason Galloway, Malte Posselt, Shai Arnon, Julia Reiss, Jörg Lewandowski & Anne L. Robertson]: IP-M, JR, JL and ALR. conceived this study and designed the sampling methodology. IP-M, JG and MP carried out the collection of samples during the field activities. IP-M, JL, JG, MP and SA were involved in the laboratory processing of geomorphology and hydrology measurements. I.P.-M. and ALR contributed to the processing of community samples (identifying and measuring). IP-M carried out the statistical analysing. Finally, IP-M wrote the manuscript, with significant contributions from JG, MP, SA, JR, JL and ALR.

Chapter 4 | Nutrients and predators control the biodegradation of emerging organic contaminants by bacteria [Ignacio Peralta–Maraver, Julia Reiss, Cyrus Rutere, Marcus A. Horn & Anne L. Robertson]: IP-M, JR, and ALR conceived this study and designed the experiments. CR and MAH carried out the isolation and preparation of the bacterial strain used in the experiments and provided microbiological advice. IP-M carried out the experimental set up and the collection of data. IP-M, JR and ALR were involved in the data analysis and interpretation of cytometer and mass spectrometer measurements. Finally, IP-M wrote the manuscript, with significant contributions from JR, CR, MAH and ALR.

Chapter 5 | Predicting leaf litter decay in the streambed: response to system compartmentalization and involvement of the whole assemblage of organisms [Ignacio Peralta–Maraver, Dan Perkins, Murray Thompson, Katarina Fussman, Julia Reiss, Anne Robertson]: IP-M, DP, MT, JR and AR conceived the ideas and designed the methodology; I P-M, AR, and MT carried out the sampling activities; I P-M and KF processed the samples; IP-M and DP analysed the data; IP-M and AR led the writing of the manuscript. All authors contributed critically to the drafts.

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Chapter 1 | Thesis structure, overall aims and questions

The ecological importance of streams and rivers on our planet exceeds any controversy.

Probably one of the most significant scientific advances in the history of freshwater research was rejecting the unidimensional concept of streams and rivers as simple pipes receiving and transporting water and solutes along the catchment to the sea. Nowadays our perspective encompasses streams and rivers as compartmentalized ecosystems in which complex multidimensional flow paths link the open channel with groundwater systems and the riparian zones (Bencala 1993). The interface of saturated sediments beneath the open channel by which this connection occurs (Fig 1.1) is known as the hyporheic zone (HZ; from the Greek *hypo* = under and *rheos* = flow or current) (Triska *et al.* 1989).

The HZ was originally proposed by Orghidan (1959), who described this interface as a discrete streambed compartment housing a distinctive biota. Since then, research into the HZ has increased exponentially, encompassing fields such as ecology, hydrology, geomorphology, biogeochemistry, environmental engineering and conservation (Dahm *et al.* 2007). Therefore, and as I discuss in chapter 2, a general definition and delineation of the HZ covering all the disciplines might be extraordinarily challenging. Still, it

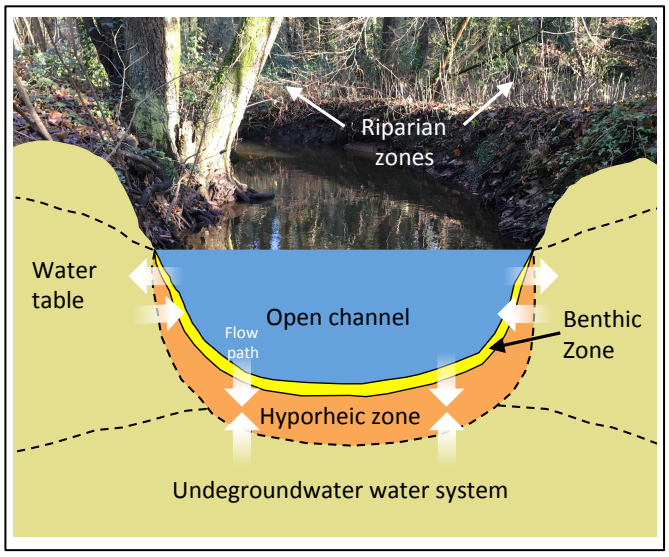

Fig 1.1. Ilustrative representation of the location of the streambed compartments. White arrows represent flow paths, which result in the hyporheic interchange.

is generally assumed that the HZ is located below the surface layer of the streambed (also known as the benthic zone; BZ) and that the size typically oscillates around the centimetre scale (although sizes of 10 m depth and 3 km width have been reported, see Stanford & Ward 1988). Even though the HZ is only a small part of any given stream its global importance as a biotope may be overwhelming in terms of size. The global surface area of streams and rivers has been estimated at around 600,000 km^2 (Battin *et al.* 2008, Downing *et al.* 2012). Just one cubic metre of streambed sediments provides a surface area of approximately 100m^2 for gravel sediments of 5 cm diameter and up to 1000m^2 for grains that are ten times smaller (Battin *et al.* 2016). When considering an average streambed of just 40 cm depth, the potential global surface area of the HZ might reach 2.6 million km^2 (surface area of the Mediterranean sea equals 2.5 million km^2).

Table 1.1. Definition of different groups inhabiting the streambed. Note these are paraphyletic groups.

Group	Definition
Biofilms	Unicellular consortia of prokaryotes (archaea and bacteria), fungi and algae (in the top sediment layers) embedded in a porous extracellular matrix.
Protozoa	Eukaryotic single cell free-living organisms such as flagellates, ciliates and amoeba.
Meiofauna	Eumetazoa invertebrates whose body size generally ranges between 0.45 and 500µm
Macroinvertebrates	Eumetazoa invertebrates whose body size is generally greater than 500µm.

The high surface area of sedimentary matrix provided by the HZ is an important habitat for a wide range of organisms. A diverse assemblage of biofilms grows attached to sediment grains and covers the cavities of the pore space. Also, protists, meiofauna and macroinvertebrates (see Table 1.1 for definition) occupy the interstitial spaces

among sediment particles in the HZ, swimming in the pore space or digging into the sediment. The assemblage of hyporheic organisms is called *hyporheos* and has its own identity in terms of composition and structure (Williams & Hynes 1974). As I will examine in detail in chapters 2 and 3, the depth gradient and the direction (and magnitude) of the surface-groundwater exchange controls species distribution, assemblage structure and ecology in the BZ and the HZ (Fraser & Williams 1998, Boulton *et al.* 1998, Dole-Oliver 1998, Miyake & Nakano 2002, Sliva & Williams 2005, Davy–Bowker *et al.* 2006, Andrushchyshyn *et al.* 2007). This water interchange is highly variable in time and space along the continuum of streams and rivers (Malard *et al.* 2002, Cardenas 2008, Dudley-Southern & Binley 2015). Consequently, the distributional limit between the benthos and the hyporheos is quite dynamic. The HZ is also colonised by organisms from adjacent environments such as stygobites from groundwater or excavating benthic biota (see Robertson & Wood 2010). In addition, the HZ may act as a refugium for benthic organisms escaping from a variety of perturbations and the pressures of biotic interactions (Williams & Hynes 1984). Therefore, categorically stating that the hyporheos form a discrete community (as an ecological entity) could be ambiguous and imprecise. Indeed the hyporheos is often inadequately described and quantified (Eichhorn 2016). Hence, there is a pressing need for quantitative approaches in order to assess the organization of streambed assemblages through the depth profile and under different hydrodynamic conditions. This will also be crucial to characterise the contribution of streambed compartments to diversity and production in the whole streambed ecosystem.

The HZ has been typically considered to be a habitat with high rates of biochemical reactions (i.e.Valett *et al.* 1996, Martí 1997, Fellows *et al.* 2006, Mulholland & Webster 2010). As a consequence of this ability, the HZ is widely

acknowledged to provide important ecosystem services and goods (Robertson & Wood 2010), including a critical role in the cycling of nutrients and organic compounds, and contaminant attenuation (i.e. McClain *et al.* 2003, Smith *et al.* 2009, Boulton *et al.* 2010, Lewandowski *et al.* 2011). Thus the HZ acts as a true bioreactor (hyporheic bioreactor) with an impressive capacity of water-purification (i.e. pollutant degradation) and organic matter recycling (i.e. leaf litter breakdown) in streams and rivers (Tank *et al.* 2010, Lewandowski *et al.* 2011). Despite the importance of such ecosystem services, mechanisms behind the functioning of the hyporheic bioreactor remain poorly studied. It has been suggested that active and diverse streambed assemblages sustain and maintain the bioreactor capacity (Krause *et al.* 2009, Bardini *et al.* 2012, Sánchez-Pérez *et al.* 2013). However, previous research has focussed on the capacity of biofilms to process dissolved nutrients and pollutants in the pore space of the streambed (Battin et al. 2003, Storey *et al.* 2004, Beaulieu *et al.* 2011, Stegen *et al.* 2016). The role of the other taxonomic groups in this biorreactor functioning (as potential bioturbators, biofilm grazers, etc.) is nearly unknown.

Ecological research in the HZ is a challenge due to the great number of variables involved in its operation. Furthermore, as I explore in chapter 2, there is a notable hierarchy of these variables, many of them being nested or correlated spatially and temporally. Its study is also becoming one of the most developed areas in freshwater science in recent years, principally due to the development and increasing accessibility of better technological approaches. Nevertheless, studies on the role of the HZ in the functioning and ecosystem service delivery of the whole river system are still maturing and remain a major research focus and challenge (Robertson & Wood 2010). Questions about the transformation of contaminants and nutrients by the hyporheos, and how hyporheic exchange flow may determine structure and energy fluxes of these

communities are still unanswered. Accordingly, holistic and truly interdisciplinary approaches at the interface of hydrology, community ecology and biogeochemistry are the only valid strategy to assess all these issues (Robertson & Wood 2010, Boulton *et al.* 2010). This thesis is an attempt to bring this gap. To do so, I adopted a logical stepwise progression integrating methods from different fields of freshwater research. In this manner, I aimed to establish solid knowledge foundations at every step before addressing more complex issues. I start with an introductory and review chapter, followed by three data chapter (two survey studies in the field and a laboratory experiment) and closing with a general discussion and concluding remarks (Fig 1.2). The main objectives and questions I will address through the following chapters are:

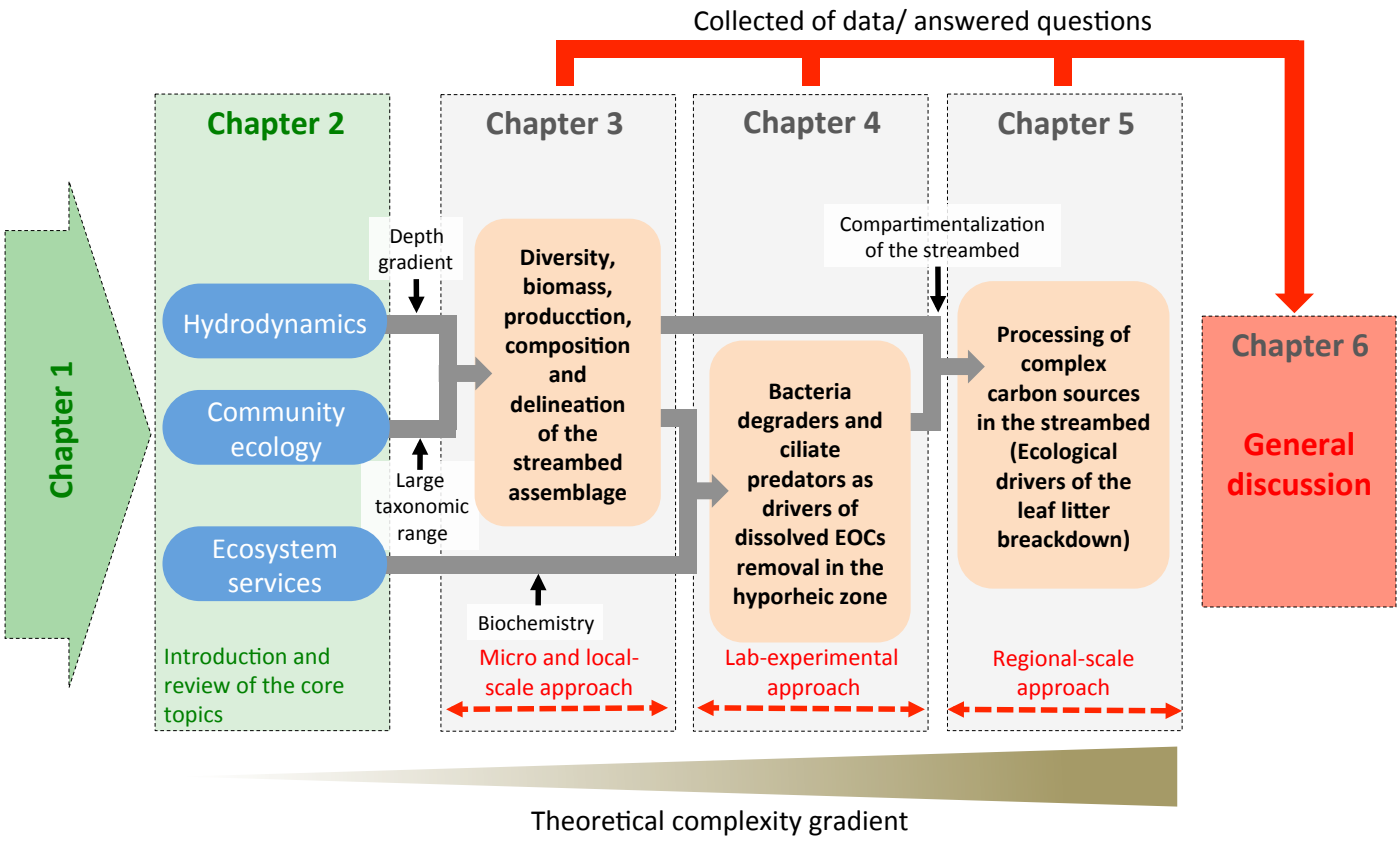

Fig 1.2. Flow chart diagram illustrating the theoretical hierarchy that this thesis will follow through the different chapters.

Chapter 2 | **Interplay of hydrology, community ecology and pollutant attenuation in the hyporheic zone**

In this chapter I review the development and current state of knowledge of the three core topics of this thesis and the interconnection between them: Hydrology, community ecology and ecosystem services (Fig 1.2). First, I examine hyporheic hydrodynamics as the engine that drives the ecological functioning of the HZ. I pay special attention to the spatial and temporal dynamics, current methodologies used to characterize the vertical flow paths of water, and lastly, its role shaping the streambed assemblage. Second, I focus on the composition, structure and ecology of the hyporheos and its importance in the biomass and production budget of the whole stream system. Using metadata analysis, I model the relationship between the biomass and production of the streambed assemblage and the depth gradient. From my models output, I discuss which explanatory variables are needed in order to improve predictability. Finally, I conceptualize the hierarchical interplay of these factors with the biorreactor ability of the HZ. Then, using this theoretical framework of knowledge I highlight the unknown mechanisms in the ecological functioning of the HZ. This chapter is therefore a detailed introduction to this thesis, which supports the importance and impact of the results achieved in later chapters.

Chapter 3 | **Environmental filtering and community delineation in the streambed ecotone**

In this data chapter I address the following questions: (**1**) *What is the effect of depth gradient and direction of vertical water exchange on diversity, biomass and productivity of streambed assemblages?* (**2**) *Is this effect similar for all the taxonomic groups?* (**3**) *Are differences in the assemblage structure large enough to distinguish*

benthos and hyporheos as discrete ecological communities? (**4**) *If this is the case, at which depth can we locate the boundary between both communities?* (**5**) *Is this limit also influenced by the vertical water exchange?*

Here, I applied a holistic and truly multidisciplinary study in order to address the hierarchical interplay between environmental filtering and community ecology the freshwater streambed. Combining methods imported from hydrology, chemical engineering and community ecology I was able to clearly distinguish between the benthos and the hyporheos. This is especially relevant within the current framework of criticism concerning the integrity of communities as real biological entities. The demarcation between benthic and hyporheic communities was influenced by the hydrodynamic conditions, the lower limit of the benthic community occurred at greater depths in downwelling (DW) than in upwelling (UW) zones.

The environmental filtering concept has been traditionally applied to determine community identity at large spatial scales; in this chapter I showed that environmental filtering also operated at a micro-scale and determined how diversity, productivity and composition of the streambed assemblage varied with depth and with the direction of vertical water exchange. Another innovative aspect of this study was that I included organisms across a wide range of body sizes (flagellates, ciliates, meiofauna and macroinvertebrates) in my analysis (most of the studies on ecology of stream bed systems focus exclusively on macroinvertebrates which are relatively large in size). As a result I was able to demonstrate that the rate at which biomass and production decreased with increasing depth differed significantly for different taxonomic groups.

Chapter 4 | **Nutrients and predators control the biodegradation of emerging organic contaminants by bacteria**

This data chapter focusses on a single question: (**1**) *How do predation and nutrient availability determine the efficiency of bacteria during contaminants degradation?*

This apparently simple question hides complex multivariable mechanisms that are difficult (if not impossible) to elucidate from survey studies in the field. Fresh water is probably the most indispensable natural resource. Therefore, taking into account the actual framework of freshwater contamination worldwide, any research assessing contaminants fate and processing in natural systems acquires a global interest. Collaborative research with state-of-the art technologies from multiple disciplines has focused on the functioning of the pore space interface in streambed sediments due to its fundamental role in the retention and attenuation of organic contaminants. However, actual knowledge of contaminant biodegradation is mainly limited to descriptive assays. Furthermore, despite acceptance that bacterial biofilms have a key role in the processing of contaminants in the pore space interface, the role of other factors in the process, such as availability of nutrients and the rest of the community (i.e. bacterial predators) has not been determined.

This chapter therefore fills a significant knowledge gap in the mechanistic understanding of the self-purifying capability of streambed systems. I experimentally assessed how dissolved organic carbon (glucose) and predator-prey interactions drive the capacity of streambed bacteria to process a model emerging organic contaminant (ibuprofen). Finally, I use my findings to develop a conceptual overview of EOCs degradation in the streambed.

Chapter 5 | **Predicting leaf litter decay in the streambed: response to system compartmentalization and involvement of the whole assemblage of organisms.**

In this last data chapter, building on research detailed in Chapters 3 and 4 I attempted to answer more complex questions: (**1**) *How does leaf litter processing differ between streambed compartments (HZ vs BZ)?* (**2**) *Is the whole assemblage of organisms (including prokaryotes, protists and eumetazoa invertebrates) involved in this important ecosystem process?* (**3**) *How is the biomass and diversity of Protists and Eumetazoa invertebrates related to leaf litter processing?* (**4**) *How are the biomass, functional diversity and metabolic capability of prokaryotes related to leaf litter processing?* (**5**) *Does the effect of the afore-mentioned biological variables on leaf litter processing depend on the streambed compartment?*

Significant advances have been made in the study of organic matter processing (Robertson & Wood 2010), however, the relationship between streambed compartmentalization (BZ *vs* HZ), the differential ecology of the benthos and hyporheos and microbial metabolism and how they drive this process remain poorly assessed. In this study I followed a regional-scale approach, including again a large range of taxonomic groups (Prokaryota, Protozoa and Eumetazoa invertebrates) and combining different methodologies to elucidate the main factors driving the leaf litter breakdown rate in the streambed. Firstly, I compared the biological variables of biomass, α-diversity, prokaryotic functional diversity and metabolic activity between the BZ and the HZ across 30 rivers in England and Wales. After describing the system, I inferred how previous variables were related with leaf litter processing by modelling the decay rate of several substrata and the leaf litter stabilisation factor. My findings

revealed that the whole assemblage contributes to the process, and that processing was notably reduced in the HZ.

Chapter 6 | General discussion

At the end of the thesis, I compile my research findings in a final framing chapter. Here I discuss how my research has answered the proposed questions in this introduction. Then, I state the main conclusions from previous chapters and their implications in the existing body of knowledge about the subject. Finally, I also discuss the implications of my thesis for future ecological studies of the streambed and next logical paths for future investigation.

1 | References

Andrushchyshyn, O. P. *et al.* Ciliate communities in shallow groundwater: seasonal and spatial characteristics. *Freshwater. Biol*. **52** 1745-1761 (2007).

Bardini, L. *et al.* Nutrient cycling in bedform induced hyporheic zones. *Geochim. Cosmochim. Acta.* **84**, 47–61 (2012).

Battin, T.J. *et al.* Contributions of microbial biofilms to ecosystem processes in stream mesocosms. Nature. **426**, 439–442 (2003). Battin T. *et al.* Biophysical controls on organic carbon fluxes in fluvial networks. *Nature Geosci*. **1**, 95–100 (2008).

Battin, T.J. *et al.* The ecology and biogeochemistry of stream biofilms. *Nature Reviews Microbiology*, **14**, 251 (2016).

Beaulieu, J.J. *et al.* Nitrous oxide emission from denitrification in stream and river networks. *Proc. Natl Acad. Sci. USA,* **108**, 214–219 (2011).

Bencala, K.E.A perspective on stream-catchment connections catchment connections. *J. N. Amer. Benthol. Soc.* **12**, 44–47 (1993).

Boulton, A.J *et al.* The functional significance of the hyporheic zone in streams and rivers. *Annu. Rev. Ecol. Evol. Syst.* **29**, 59-81 (1998).

Boulton, A.J. *et al.* Ecology and Management of the hyporheic zone: stream-groundwater interactions of running waters and their floodplains. J. N. Amer. Benthol. Soc. **29**, 26–40 (2010).

Cardenas, M.B. (2008). The effect of river bend morphology on flow and timescales of surface water–groundwater exchange across pointbars. *J. Hydrol.* **362**, 134-141.

Dahm, C.N. *et al.* in Methods in Stream Ecology. 2nd Edddition (eds Hauer F. R. & Lamberti G. A.) 119−142 (Academic Press, San Diego 2007).

Davy–Bowker, J. *et al.* The distribution of benthic and hyporheic macroinvertebrates from the heads and tails of riffles. *Hydrobiologia*, **563**, 109-123 (2006).

Dole-Oliver, M.J. Surface water-groundwater exchanges in three dimensions on the backwater of the Rhône River. *Freshwater. Biol*. **40**: 93-109 (1998).

Downing, J. *et al.* Global abundance and size distribution of streams and rivers. *Inland Wat*. **2**, 229–236 (2012).

Dudley-Southern M., & Binley A. Temporal responses of groundwater-surface water exchange to successive storm events. *Water Resour. Res.* **51**, 1112-1126 (2015).

Eichhorn, M. *Natural systems: the organisation of life.* (John Wiley and Sons, Chichester, 2016).

Fellows, C. S. *et al.* Coupling nutrient uptake and energy flow in headwater streams. *Ecosystems*, 9*,* 788−804 (2006).

Fraser, B.G. & Williams, D. D. Seasonal boundary dynamics of a groundwater/surface-water ecotone. *Ecology,* **79**, 2019-2031 (1998).

Krause, S. *et al.* Hyporheic hydrology: interactions at the groundwater-surface water interface. *Hydrol. Process.* **23**, 2103–2107 (2009).

Lewandowski, J. *et al.* Fate of organic micropollutants in the hyporheic zone of a eutrophic lowland stream: Results of a preliminary field study. *Sci. Total Environ*. **409**, 1824–1835 (2011).

Malard, F. *et al.* A landscape perspective of surface–subsurface hydrological exchanges in river corridors. *Freshw. Biol.* **47**, 621-640 (2002).

Martí, E. Pre-and post-flood retention efficiency of nitrogen in a Sonoran Desert stream. *J. N. Am. Benthol. Soc*. **16**, 805−819 (1997).

McClain, M.E. *et al.* Biogeochemical hot spots and hot moments at the interface of terrestrial and aquatic ecosystems. *Ecosystems*, **6**, 301–312 (2003).

Miyake, Y. & Nakano, S. Effects of substratum stability on diversity of stream invertebrates during baseflow at two spatial scales. *Freshwater. Biol*. **47**, 219–230 (2002).

Mulholland, P.J. & Webster, J.R. Nutrient dynamics in streams and the role of J-NABS. *J. N. Amer. Benthol. Soc*. **29**, 100–117 (2010).

Orghidan, T. Ein neuer Lebensraum des unterirdischen Wassers: der hyporheische Biotop. *Arch. Hydrobiol*. **55**, 392−414 (1959).

Robertson, A. L. & Wood, P. J. Ecology of the hyporheic zone: origins, current knowledge and future directions. *Fundam. Appl. Limnol./Arch. Hydrobiol*, **176**, 279-289 (2010).

Sánchez-Pérez, J.M. *et al.* Role of the hyporheic heterotrophic biofilm on transformation and toxicity of pesticides. *Ann. Limnol. - Int. J. Lim.* **49**, 87–95 (2013).

Sliva, L. & Williams, D.D. Responses of hyporheic meiofauna to habitat manipulation. *Hydrobiologia*, **548**, 217-232 (2005).

Smith, J.W.N. *et al.* Pollutant attenuation at the groundwater – surface water interface: A classification scheme and statistical analysis using national-scale nitrate data. J. Hydrol. **369**, 392–402 (2009).

Stanford, J.A. & Ward, J.V. The hyporheic habitat of river ecosystems. *Nature*, **335**, 64−66 (1988).

Stegen J.C. *et al.* (2016). Groundwater–surface water mixing shifts ecological assembly processes and stimulates organic carbon turnover. *Nat. Commun.* **7**, 11237.

Storey, R.G. *et al.* Nitrogen processing in the hyporheic zone of a pastoral stream. *Biogeochemistry*, **69**, 285-313 (2004).

Tank, J.L. *et al.* A review of allochthonous organic matter dynamics and metabolism in streams. *J. N. Am. Benthol. Soc*. **29**, 118-146 (2010).

Triska, F.J. *et al*. Retention and transport of nutrients in a third-order stream in Northwestern California: Hyporheic processes. *Ecology*, **70**, 1893−1905 (1989).

Valett, H.M. *et al.* Parent lithology, surface–groundwater exchange, and nitrate retention in headwater streams. *Limnol. Oceanogr.* **41**, 333−345 (1996).

Williams, D.D & Hynes, H.B.N. The occurrence of benthos deep in the substratum of a stream. *Freshw. biol*. **4**, 233−256 (1974).

Chapter 2 | Interplay of hydrology, community ecology and pollutant attenuation in the hyporheic zone

Ignacio Peralta–Maraver[1], Julia Reiss[1] & Anne L. Robertson[1]

[1]Department of Life Sciences, Roehampton University, London, UK.

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1 | Abstract

1) We describe the hierarchical interplay of hydrology, hyporheic ecology and transformation of nutrients and pollutants in the hyporheic zone (HZ). The exchange of water between the surface–subsurface generates the hyporheic exchange flow: the engine that drives the ecological functioning of the HZ. The magnitude and direction of hydrological fluxes in the HZ follow complex spatial patterns, strongly influenced by the temporal dynamics of surface flow in rivers.
2) The direction and magnitude of hydrological fluxes also shapes the structure of hyporheic communities (hyporheos). During surface disturbances such as flooding or drought, benthic organisms may also use the HZ as a refuge, although the importance of this role is debated.
3) Streambed organisms differ in their ability to colonise the HZ depending on the biological traits they possess. The reduction in oxygen concentration and pore size with increasing sediment depth imposes a limit on the distribution of macroinvertebrates, which are replaced by a suite of smaller organisms (meiofauna and protists) at deeper sediment layers. Therefore, a concomitant reduction in net biomass and productivity might be expected through depth. However, only a few studies have assessed the contribution of the hyporheos to whole system production, and they have focused only on the fraction of relatively large organisms.
4) The bioreactor ability of the HZ to transform nutrients and pollutants is an important ecosystem service sustained by the life activities of hyporheos. Biofilms have the key role in this process due to their capacity to metabolize a wide range of dissolved compounds, including emerging pollutants. However, the residence time of water in pore sediments (resulting from hyporheic exchange flow) and the rest of the community (constantly reworking the sediments and grazing biofilms) are indirectly involved.

macroinvertebrates | meiofauna | biofilms | micropollutants | bioremediation | ecosystem services

2 | Introduction

In most lotic systems, the surface water of the open channel is connected to groundwater systems via the riverbed sediments. As a result, there is a bi–directional exchange between the groundwater and the surface water along the continuum of stream and rivers (Bencala 1993). The volume of the sediments in which stream water mixes with groundwater is known as the hyporheic zone (HZ). HZ functioning in the context of the whole–river ecosystem has been studied by researchers belonging to many different disciplines and as a result selecting a single inclusive definition for the HZ is difficult (Bencala 2000). Traditionally its definition has depended on the discipline–specific interest in hyporheic processes (Tonina & Buffington 2009) (Table 2.1). For example, in Geochemistry, the HZ is defined as the volume of sediment containing a specified percentage of surface water, while in Biology it is described as the volume of sediments housing a characteristic hyporheic community (Tonina & Buffington 2009). These differences in definition and extent have important implications. However, the fundamental concept behind all definitions is that water exchanges between the open channel and the groundwater systems.

Recently, Ward (2016) proposed a more flexible and cross–disciplinary definition (Table 2.1). A key idea from this definition of the HZ is the importance of the temporal scale relevant to the processes of interest. In fact, flow paths and the rates of water exchange through the HZ are strongly influenced by the temporal dynamics of surface flow in rivers. This is especially evident on a seasonal scale. Despite the dynamics of rivers, seasonality may result in a set of drastic changes in water flow conditions (Gasith & Resh 1999) and determine the location and extent of the HZ (Wondzell 2015). Nonetheless, the HZ buffers the amplitude of this variation, acting as a potential refuge of riverbed biota during adverse conditions (Maazouzi *et al.* 2017). This has important

implications for variations in the composition and abundance of organisms throughout the year (Stubbington *et al.* 2009). The HZ harbours diverse and productive communities whose distribution and composition is strongly correlated with the direction and magnitude of hydrologic fluxes (Standley & Boulton 1993, Olsem & Townsend 2003). These hyporheic communities or hyporheos are composed of microbial biofilms (Singer *et al.* 2006) (bacteria and fungi existing in an exocellular matrix), protists (mainly ciliates, flagellates and amoebae) and invertebrates. These groups differ notably in their biological traits and ability to colonize the riverbed, shaping the budget of biomass and secondary production in the HZ.

The HZ is a mechanical filter mediated by the pore space of sediments and water flows and a biogeochemical filter controlled by biological and chemical processes (Boulton *et al.* 2010). As a result, the HZ provides an important ecosystem service by acting as a bioreactor (hyporheic bioreactor, Table 2.1) with an impressive self–purification capacity, and a barrier against contamination of aquifers, which is essential in the supply of water for human consumption (Lewandowski *et al.* 2011). Thus the HZ of streams and rivers has a critical role in the flows of biomass and energy, cycling of nutrients and pollution attenuation (McClain *et al*. 2003, Boulton *et al.* 2010, Robertson & Wood 2010). A large body of literature describes the nitrogen, phosphorus and organic carbon attenuation in the HZ of streams and rivers (i.e. Harvey *et al.* 2013, Aubeneau *et al* 2015, Stegen *et al* 2016, Liu *et al.* 2017). However, there is little literature describing the fate and removal rates of the emerging micropollutants (Table 2.1) in lotic systems (Lewandowski *et al.* 2011, Köhler & Triebskorn 2013), making understanding of the processing of these compounds by the bioreactor a major remaining challenge in ecology.

The role of the hyporheic bioreactor in the whole river system might be seen as the 'rivers liver' (Fischer *et al.* 2005). The HZ has an important role in the production, metabolism, exchange and transformation of dissolved compounds, and health of the whole ecosystem. Here we describe the hierarchical relationship between hyporheic exchange flow, community ecology, and pollutant attenuation of the HZ. These subjects have been mainly assessed separately in discipline specific studies but they are intimately connected and together drive the functioning of the hyporheic bioreactor.

Table 2.1. Glossary of terms

Term	Definition	Reference
Hyporheic zone (Original definition)	The interstitial habitat beneath a stream, bordered by the surface water above and by the true groundwater below	Orghidan (1959)
Hyporheic zone (Geochemical definition)	The volume of sediment containing a specified percentage of surface water	Tonina & Buffington (2009)
Hyporheic zone (Hydrological definition)	The volume of sediment where water exchangebetween open channel and groundwater occur as a result of streambed pressure gradients and hydraulic conductivity	Tonina & Buffington (2009)
Hyporheic zone (Biological definition)	The volume of sediments housing a characteristic hyporheic community. This community can be defined as occasional users or permanent users	Tonina & Buffington (2009)
Hyporheic zone (Integrative definition)	Any location meeting four key criteria: [1] Saturate surface. [2] Existence of flow path that originate from and return to surface water. [3] Interaction with the stream occurs within a temporal scale relevant to the processes of interest. [4] Processes of interest occur continuously from the subsurface to the groundwater continuum.	Ward 2016
Hyporheos	The biota occupying saturated interstitial spaces below the stream surface (benthic zone)	Standley & Boulton (1993)
Upwelling (UW) zone	High–pressure areas in riverbed, where surface water comes out from HZ to the open channel	Luo *et al.* (2014)
Downwelling (DW) zone	Low–pressure areas in riverbed, where surface water enter in the HZ	Luo *et al.* (2014)
Hyporheic exchange flows	Strength and direction of the water mass through the sediment pore spaces in the HZ, resulting from the alternation of UW and DW zones	This assay

Continue table 2.1

Micropollutants	A vast and expanding array of emerging contaminants (including pharmaceuticals, personal care products, steroid hormones, industrial chemicals and pesticides) commonly present in waters at trace concentrations, ranging from a few ng/L to several μg/L.	Luo *et al.* (2014)
Hyporheic bioreactor	Active biological system in which the transformation of chemical compounds occurs as result of the hyporheos life activities or the active substances they produce.	McClain *et al.* (2003)
Residence time	Hydrodynamic retention time in the HZ during which biogeochemical processing of dissolved solutes occur	Tonina & Buffington (2009)

3 | Hyporheic hydrodynamics: the motor of hyporheic zone ecology

The water exchange between the open river channel and a groundwater system generates the hyporheic exchange flow and strongly influences the whole ecosystem by determining the transport of solutes between compartments (Ward *et al.* 2012) and the chemistry of stream water (Bencala 1993, Duff & Triska 2000). Streambed sediments are a porous medium through which exchange of water occurs. Thus, the hyporheic exchange and the flow paths throughout sediments could be theoretically studied by applying Darcy's Law, as the product of hydraulic gradient and hydraulic conductivity (Jones & Holmes 1996). However, hyporheic exchange flow does not show a uniform pattern along the river. The flow between surface and groundwater follows complex dynamics (Rutherford & Hynes 1987), in which upwelling (UW) and downwelling (DW) zones occur alternately (White *et al.* 1990). Thus, as a result of this mosaic of physical features, sediment conditions may change substantially even at centimetre scales (Boano *et al.* 2014). In addition, temporal dynamics from daily to seasonal processes result in change of hyporheic flow through time (Wondzell 2011).

Streambed topography from sediment–scale (ripples or pebbles) to larger geomorphologic features (i.e., riffle–pool sequence, steps), is the primary control of hyporheic exchange (Valett *et al*. 1993, Maddok 1995, *Dahm et al.* 1998, Calver 2001, Ward *et al.* 2012, Gomez–Velez *et al*. 2014). These features act as obstacles to the water flow along the open channel, extending the HZ both vertically and laterally from the stream (Harvey & Bencala 1993), and generating sequences of DW and UW zones (Savant *et al.* 1987, Hendricks & White 1991, Harvey & Bencala 1993). For example, within a single stream riffle, surface water enters the HZ at the beginning of the riffle (DW zone) and returns to the open channel at the end of it (UW zone) (Hendricks 1993, Hendricks & White 1991).

Hyporheic exchange flows are generally faster in headwater streams, which typically have shallow and steep reaches with cobble– and gravel–bed sediments (i.e. they are quite porous). Hyporheic exchange is progressively slower, deeper and more complex as riverbed sediments become finer (less porous) in slower flow zones (Buffington & Tonina 2009). Nevertheless, quantifying this exchange is complicated because streambed materials range from relatively homogeneous, to cases where the range of sediment sizes (and therefore the hydraulic conductivity) exceeds six orders of magnitude (Calver 2011).

In recent years a wide range of available sampling techniques has been developed to determine the heterogeneous interactions between groundwater and surface water (Kalbus *et al.* 2006). These methods range from direct measurements of water flux across the groundwater–surface water interface (i.e. seepage meter; Lee 1977), to indirect techniques such as heat tracer methods, mass balance approaches or mathematical modelling (Kalbus *et al.* 2006). Until recently, these were almost exclusively employed in studies of hydrology and engineering (Boulton *et al.* 2010).

However, understanding the importance of hyporheic exchange flow as a controlling factor of hyporheic communities and biogeochemical processes has led to increased implementation of hydrodynamic analysis in recent ecological studies of the HZ (Standley & Boulton 1993, Hendricks 1993, Boulton 1993, Schmid–Araya 1998, Miyake & Nakano 2002, Malard *et al.* 2003, Davy–Bowker *et al.* 2006, Kasahara *et al.* 2009, Robertson & Wood 2010). One interesting strategy to address these issues, which has been widely used in hydrological studies, is to implement time–series analysis of streambed profiling–thermal records (i.e. Hatch *et al.* 2006, Keery *et al.* 2007, Irvine & Lautz 2015, Irvine *et al.* 2015). These methods are based on quantifying changes in phase and amplitude of temperature variations between pairs of subsurface sensors through depth (Hatch *et al.* 2006). Nevertheless, results exclusively from these methods are often limited and contradictory (Shanafield *et al.* 2011, Briggs *et al.* 2014).

4 | Seasonality in flow exchange and its effect on riverbed communities

Streams and rivers are dynamic ecosystems par excellence. The incessant open channel flow produces continuous movements of substrata, changes streambed topography and reorganizes the morphology of the channel boundaries. In this manner, the temporal dynamics of surface flow in a lotic ecosystem is closely related to the spatial heterogeneity of flow paths and rate of exchange through the HZ at all scales. The multiple temporal and spatial scales and the rate of exchange collectively define the hyporheic residence time of water (Buffington & Tonina 2009). The residence time is an important property of the HZ, because most of the biogeochemical processes that occur in sediments depend on the rate of water flow through them (Duff & Triska 2000, Mulholland & DeAngelis 2000). Furthermore, the temporal dynamic that affects the hydrological exchange also produces fluctuations of HZ boundaries (Gibert *et al.* 1990)

and this variation in the size of the HZ determines its influence on both the open channel and the underlying groundwater (Vervier *et al.* 1992).

Seasonality in rivers is an extreme example of the temporal variation of the discharge of the open channel (e.g. due to snowmelt or the alternation between dry and rainy seasons). Accordingly, these changes alter the flow exchange patterns in the HZ (Kalbus *et al.* 2006) and may act as disturbance events for sediment organisms (Townsend *et al.* 1997, Robertson 2000). These potential disturbances may be reduced in the HZ due to its ability to maintain humidity after surface drying and remain stable during floods (Robertson & Wood 2010). Thus the HZ might serve as a refuge for the local biota during disturbances events enabling recolonization of the surface once the disturbance ends (Williams & Hynes 1974, Dole–Olivier 2011). The HZ can also act a refuge for the early instars of some macroinvertebrates due to the more stable environmental conditions and reduced predator pressure (Williams 1984). Nevertheless, the importance of the HZ as a refuge is debated (Robertson & Wood 2011), because some studies found no evidence of HZ refuge use by aquatic invertebrate fauna (Boulton *et al.* 2002, Olsen & Townsend 2003, James *et al.* 2008). In contrast, the importance of the HZ as a refuge might be more evident in seasonal intermittent streams. These systems are common worldwide and support diverse communities of aquatic organisms including many taxa that survive in dry riverbeds and/or rapidly recolonize when water returns (Stubbington & Datry 2013, Datry *et al.* 2014). Indeed, the influence of drought may be even more intense in streams that lack a marked seasonally (López–Rodríguez *et al.* 2012) (unpredictable intermittent streams). This is the case for some Mediterranean streams with supra–seasonal drought, where many organisms that survived in the HZ during the dry season recolonized the stream during the first month of the wet season (López–Rodríguez *et al.* 2012). Use of the HZ as a

refuge is not exclusive to large biota, it occurs across a wide range of organism size. Febria *et al.* (2012) observed that biofilms also use hyporheic sediments as a refuge from desiccation, mainly transported by hydrological pathways through the sediments. During periods of drought, the HZ supports bacteria associated with the infiltration of water and the creation of microhabitats in the sediment; when interstitial pore spaces become filled with water during flood events, HZ and the surface become connected allowing bacteria recolonization (Febria *et al.* 2012).

Another factor that markedly affects the hydrology of streams and rivers is the seasonal change of in–stream vegetation cover. In–stream macrophytes are typically abundant in many lotic ecosystems during spring and summer; altering river flow and trapping sediments (Champion & Tanner 2000, Dodds & Biggs 2002). In–stream vegetation may reach from 0% to over 70% of spatial coverage between winter and summer in European rivers (Cotton *et al.* 2006). This increase in macrophytes is coupled with a drastic reduction of the open channel flow velocity and the deposition of fine sediments (Cotton *et al.* 2006). Nevertheless, to our knowledge, there is no published research that assesses the effect of in–stream macrophyte dynamics on hyporheic flow and hyporheic communities. In addition, daily fluctuations in stream flow may also be caused by evapotranspiration of vegetation (including riparian vegetation) and it has been hypothesized that this transpiration enlarges hyporheic flow paths during the day and decreases them at night (Wondzell *et al.* 2010).

5 | Hyporheic zone as a budget of biomass and production in streams and rivers

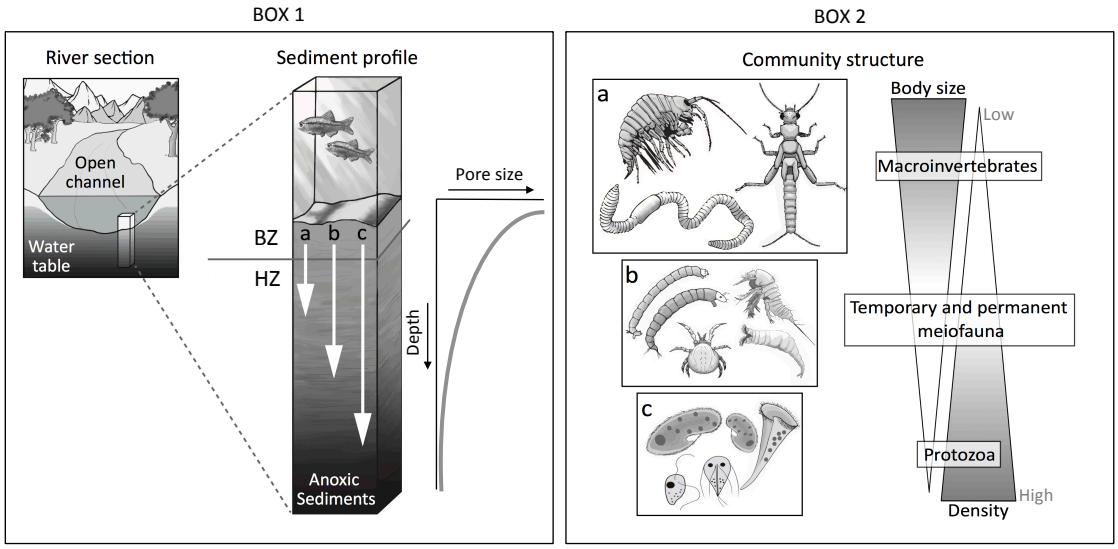

Fig 2.1. BOX 1: Scheme of the streambed community distribution throughout the depth profile in relation to pore size and redox potential. Arrows represent colonization depth of large macroinvertebrates (a), temporary and permanent meiofauna (b) and Protozoa (c). Also shown is the theoretical boundary between benthic zone (BZ) and hyporheic zone (HZ) as the colonization limit between benthos and hyporheos. The grey/black scale in the sediment profile indicates the redox potential with dark black as strongly anoxic conditions. BOX 2: Body size and density distributions of different groups in the community structure. Organisms are not drawn to scale.

Defining system boundaries is an important aspect of the study of ecological processes (Smock *et al.* 1992). Streams and rivers have been viewed traditionally as having three interactive spatial compartments: firstly the open channel and benthic zone (BZ), secondly the HZ and thirdly the riparian zone (Cummins *et al.* 1983, Ward 1989) and each compartment could play a different role depending on the ecological process under study. Secondary production is a useful measure of the energy flux (as biomass) produced by heterotrophic organisms over time and space (Benke & Huryn 2007). However, there are only a few studies, which have determined the relative contribution of the HZ compartment to whole system production (i.e. Smock *et al.* 1992, Collier *et al.* 2004, Wright–Stow *et al.* 2006, Reynolds & Benke 2012). Previous studies have defined the top 0 to 5–10 cm of sediments as the benthic zone (BZ), and lower depths as

HZ despite its proposed biological definition (Table 2.1). In order to accurately define the limits of the HZ compartment, a small–scale approach across a depth gradient is needed (i.e. variation in assemblage structure at centimetre scale, determination of distributional limits of characteristic taxa from compartments).

Some studies have shown that invertebrate assemblages comprising a suite of relatively few and large individuals near the surface are replaced by numerous but small–bodied organisms with increasing depth (Schmid–Araya 1994, Stead *et al.* 2004). This is because the taxa in the hyporheos differ in their ability to penetrate the HZ depending on their biological attributes (Descloux *et al.* 2004, Nogaro *et al.* 2009, Robertson & Wood 2010). The reduction in oxygen concentration and pore size due to sediment agglomeration along the depth gradient (Fig 2.1) limits the distribution of large macroinvertebrates with higher metabolic rates (Maridet & Philippe 1995, Strayer *et al.* 1997). As a result, the density of meiofauna (microscopically small metazoans) and protists should increase with depth (Fig 2.1). In fact, the reduction in density of large organisms through depth has been broadly reported as a general pattern in studies of riverbed communities (i.e. Dole–Olivier *et al.* 1994, Maridet & Philippe 1995, Davy–Bowker *et al.* 2006, Marchant 1995, Pacioglu & Robertson 2017). Accordingly, it might be hypothesized that the depth gradient, as a set of different physicochemical factors, is also a key variable causing the decline of biomass and secondary production of riverbed systems. Our metadata analysis of invertebrate communities from different river systems [using data from Smock et al (1992) and Reynolds and Benke (2012)] corroborates this prediction, showing a negative and significant effect of depth both on biomass and secondary production (Fig 2.2; an explanation of these analyses is available in appendix 1). However, despite its significance, the regression model explains only a small part of the observed variation (marginal $R^2 = 11\%$, and conditional

R^2 = 54%, see appendix 1) and so other variables must be important. This meta–analysis is limited to macroinvertebrate communities because studies of changes in biomass and secondary production along the depth gradient have focused exclusively on large size organisms ignoring most meiofauna and the Protozoa.

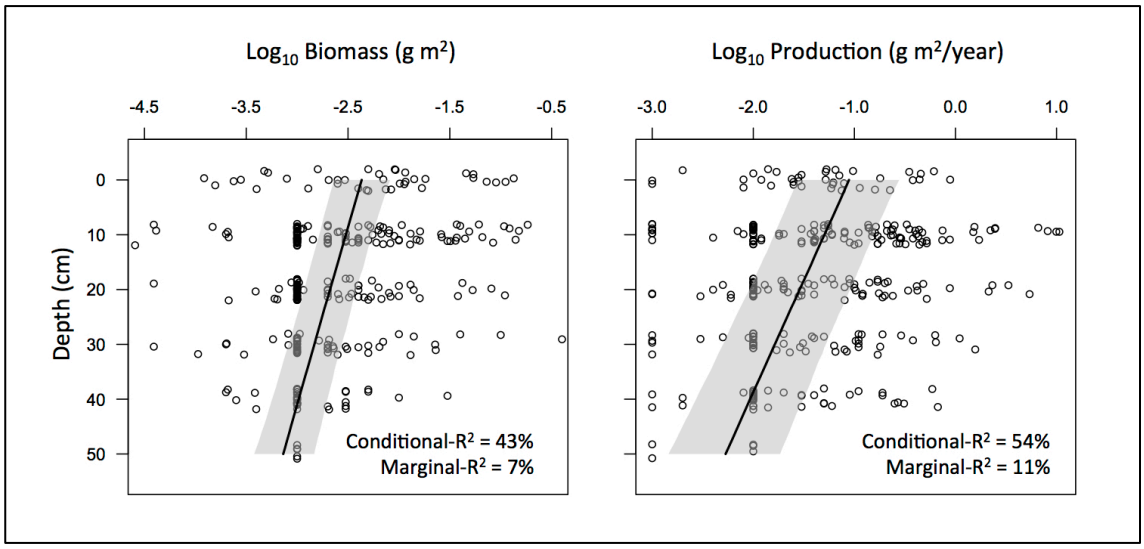

Fig 2.2. Depth–related biomass and production of invertebrates based on reported values from Smock *et al.* (1992) and Reynolds and Benke (2012). Predictions (black line) represent the $\log_{10}$ biomass and production values and are derived from the linear mixed models explained in the Appendix, with 95% credible intervals (shaded grey). Open circles represent the $\log_{10}$–transformed values per taxa. Note that even though the model detected the negative effect of depth on the responses, the marginal R^2 is low. This explains the large dispersion in the scatter plot and evidences the necessity of including more factors (i.e. taxonomic group, hydrological conditions in future analyses).

Remembering that taxa comprising the hyporheos differ in their ability to penetrate into the HZ depending on their size, we expect a significant interaction between depth and size group (flagellates, ciliates, meiofauna and macroinvertebrates). Including these groups and their interaction with depth, hydrology and sediment characteristics in future research studies would notably improve predictive modelling and compartment comparisons. This is of particular interest because it has been

proposed that the bioreactor ability of the HZ is sustained and maintained by diverse and active hyporheic communities (Krause *et al.* 2009). Accordingly, it might be predicted that hot spots of nutrient and pollutant transformation may coincide with areas containing higher biomass and secondary production rates. It could also be expected that the role of different organisms in the bioreactor would vary following the depth gradient. For example, bioturbation and bioirrigation resulting from life activities of relatively large burrowers (such as Chironomidae larvae, Ephemeridae nymphs or Oligochaeta), would be more important in the benthic zone and upper layers of the HZ than in deeper layers. These processes promote sediment permeability, respiration of freshwater sediments and bacterial activity (Bertics & Ziebis 2009, Hölker *et al.* 2015, Baranov *et al.* 2016), and so have a great impact on water biogeochemistry (Morad *et al.* 2010).

6 | The hyporheic bioreactor

Flow exchange and pore water chemistry of the HZ can be also affected by anthropogenic activity, typically with negative effects on ecosystem health. A common alteration that occurs in rivers across the world is the artificial water input from wastewater treatment plants (WWTPs) (Carey & Migliaccio 2009) due to which many rivers receive permanent or pulsed inputs of nutrients (i.e. organic carbon, nitrate, phosphate) and other pollutants (i.e. pesticides) as a result of human activities (Boyer *et al.* 2006, Mulholland *et al.* 2008). However, once these compounds penetrate into the sediments as a consequence of the hydrological patterns, they may be transformed into oxidized or reduced substances by metabolic reactions, mediated by active and productive hyporheic communities (Krause *et al.* 2009, Bardini *et al.* 2012, Sánchez–Pérez *et al.* 2013). Accordingly, the HZ acts as a true water–purifying bioreactor in

which microbial biofilms play an important role. Hyporheic biofilms are dominated by highly diverse bacteria and archaea communities embedded in the same matrix of polysaccharides (Battin *et al.* 2016). This results in the coexistence of a great range of operational taxonomic units (Zeglin *et al.* 2015), diverse metabolic capabilities (Singer *et al.* 2010, Battin *et al.* 2016) and sites of high enzymatic activity (Romaní *et al.* 2008). Thus, in–stream biofilms are important components of the global biogeochemical fluxes of carbon, nitrogen and phosphorous (Mulholland *et al.* 2008, Battin *et al.* 2008, Boano *et al.* 2014). The supply of nutrients is assumed to be a limiting factor in determining the biomass, activity, and physiology of subsurface microbial communities (Bengtsson 1989). Thus bacterial biomass and metabolic activity should be significantly greater under situations of higher input of DOC (Foulquier *et al.* 2011). In addition, water is pumped in and out of the HZ and riparian zone on a daily cycle because water stage variation, generates large hydraulic gradients and enhanced mixing in highly regulated rivers (Gerecht *et al.* 2011). These daily fluctuations of the river stage stimulate bacterial respiration and organic carbon turnover (Liu *et al.* 2017).

7 | Micropollutants, the new challenge for the hyporheic bioreactor

Recent research has shown that nitrogen, phosphorus and organic carbon are important pollutants in the HZ and aquatic ecosystems generally (Lewandowski & Nützmann 2010, Bardini *et al.* 2012, Maazouzi *et al.* 2013, Harvey *et al.* 2013). However, surface water systems and their interactions with groundwater systems are increasingly under pressure from a new group of chemicals; the micropollutants (Langenhoff *et al.* 2013, Luo *et al.* 2014). The occurrence of micropollutants (such as pharmaceutical and personal care products; i.e. ibuprofen or antibiotics) due to WWTPs inputs has greatly increased in stream and rivers. The concern about their presence is

mainly related to potential adverse effects on environmental systems (i.e. bioaccumulation) and to human toxicology (i.e. aquifer contamination) (Hernández Leal *et al.* 2010, Langenhoff *et al.* 2013). Furthermore, the chronic low–level antibiotic exposures detected in aquatic systems acts as a selective process on bacteria communities (Hirsch *et al.* 1999, Yang & Carlson 2003). Thus, differential antibiotic tolerance of a bacterial community may produce a shift in the biofilm structure (composition, richness, density), affect the spatial distribution of members of the community (Roose–Amsaleg & Laverman 2016) and change the ability of biofilms to conduct ecosystem services (e.g. reducing denitrification processes due to deleterious impacts on denitrifying bacteria; Costanzo *et al.* 2005). However, in some cases, micropollutants can be efficiently attenuated along flow paths in the HZ (Lewandowski *et al.* 2011). Indeed, some of these compounds (i.e. diclofenac, bezafibrate, ibuprofen, and naproxen) are more efficiently transformed in river sediments than in WWTPs by biofilms (i.e. McClain *et al*. 2003). This is mainly related to the higher diversity of microbial communities in environmental systems. In addition, water residence times in the HZ are longer than in the open channel and surface sediments (and WWTP), allowing more efficient biodegradation processes (Lewandowski *et al.* 2011).

Notwithstanding the role of the biofilm is recognized in pollutant attenuation, more complex questions behind this ecological process remained unanswered (i.e. the role of the rest of the community). Furthermore, it is also important to consider the hierarchical interaction between hydrological patterns and biogeochemical processes in the study of the nutrient and pollutant breakdown in the HZ. Hydrodynamics throughout the sediments may induce two opposite effects on solute reactions. Higher inward water fluxes lead to a larger input of substances into the HZ enhancing reaction rates, but also the hyporheic microbiota has less time to perform biogeochemical reactions due to the

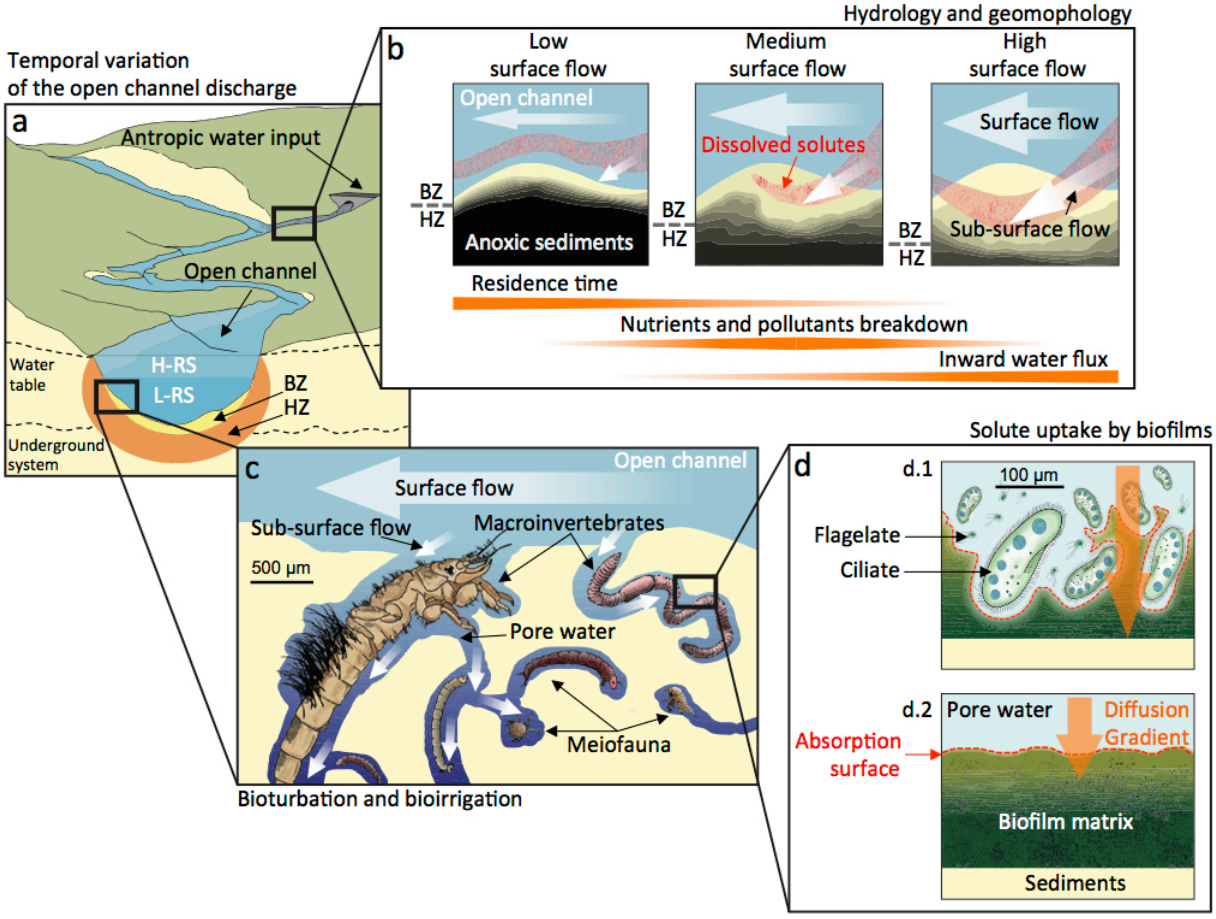

Fig 2.3. The ability of the HZ to process dissolved solutes is mediated by a hierarchical interaction between hydrological patterns and community ecology. (**a**) Daily and seasonal fluctuations between the high river–stage (H–RS) and low river–stage (L–RS) cause variation in the open channel discharge and the water table stage (dashed lines in the figure represent high and low water table stage). (**b**) The increase in the surface flow promotes more input of water (higher sub–surface flow) and input of dissolved solutes into the HZ, but also the residence time of water in the sediments decreases. The grey/black scale indicates the redox potential with dark black as strongly anoxic conditions. (**c**) Life activities of macroinvertebrates and meiofauna result in bioturbation and biorrigation phenomena in the streambed sediments, causing the occurrence of preferential flow paths and increasing permeability locally. (**d**) Protists grazing on biofilms increase its absorption surface. As a result, dissolved solutes diffusion gradient is higher in presence of grazers (**d1**) than in their absence (**d2**).

lower residence times of the compounds in the sediments (Bardini *et al.* 2012) (Fig 2.3b). These mechanisms will become even more complex when we recall that hydraulic conductivity may also be affected by the action of the hyporheos. Growth of biofilm matrices in the sediment pores reduces permeability and increases residence times of water in the HZ (Findlay & Sobczak 2000, Battin *et al.* 2003). In addition,

biofilm theory holds that uptake of solutes is diffusion–limited by the thickness of the biofilm polysaccharide matrix (Gantzer *et al.* 1988). Before assimilation, solutes must pass first from the pore water to the biofilm surface (external mass transfer) and then through the biofilm matrix to the cells (internal mass transfer) (Battin *et al.* 2003). However, sediments are constantly being reworked, increasing the sediments permeability locally (Boulton 2000). Life activities of macroinvertebrates, meiofauna and protists (e.g. ciliates and flagellates) in the HZ (digging, removing the sediments and grazing on biofilms) result in preferential flow paths, increasing biofilm surface and boosting bacterial densities (Danielopol 1976, Boulton 2000, Battin *et al.* 2003, Mermillod–Blondin *et al.* 2003), acting as ecosystem engineers (Fig 2.3c, d). Thus, the net effect on breakdown rates depends on the balance between all these opposing factors (Arnon *et al.* 2007, Cardenas 2008, Bardini *et al.* 2012). In this manner, mechanistic understanding of biofilms function can be acquired only through carefully designed experiments under well–defined conditions and appropriate cultivation techniques (Singer *et al.* 2006). Accordingly, controlled experiments are needed to explore the underlying causal mechanisms that generate the patterns seen in reach–scale descriptive surveys (Olsen & Townsend 2003).

9 | Acknowledgements chapter 2

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10 | Appendix chapter 2

Appendix 2.1. Suplementary methods

Biomass and secondary production data along the depth gradient were extracted from reported values in Smock *et al.* (1992) and Reynolds and Benke (2012). A similar sampling method (sediment corers) and spatial scale resolution were used in both studies. Smock *et al.* (1992) reported biomass and secondary production of the whole community of macroinvertebrates in a sandy–sediment river (Buzzards Branch). While, Reynolds and Benke (2012) measured genus–specific biomass and secondary production of chironomid larvae (Diptera) assemblage (composed of 26–31 genera) along a hyporheic gradient, comparing gravel–cobble of a high–alkalinity stream (Hendrik Mill Brach) with sandy and mud–silt habitats of a low–alkalinity stream (Payne Creek). The chironomid assemblages were reasonably similar between studied streams. Furthermore, chironomid larvae are usually the dominant and richest invertebrate group in freshwater benthic habitats (Ferrington *et al.* 1996). Hence, it could be assumed as a good model group to infer general production patterns of invertebrates.

Two linear mixed effect models (LMMs) were applied to test the effect of, depth (continuous covariant) on biomass and production (responses). Responses were Log_{10} transformed to solve heterogeneity in the residuals. The Akaike Information Criterion (AIC) was then used to find the most parsimonious model by combining the fixed term (depth) and potential random effects (i.e. studied site, differences between taxa). As a result, biomass and production models included depth (single covariate), study site (studied river as random intercept) and the interaction between depth and taxa (random slope) as effective parameters:

$$Log_{10}(\hat{y}_{ij}) = \beta_0 + a_{site} + \beta_1 \times Depth_j + b_i \times Depth_j + \varepsilon_{ij}$$

$$\varepsilon_{ij} \sim Norm(0, \sigma^2)$$

$$a_{site} \sim Norm(0, \sigma^2_{site})$$

$$b_i \sim Norm(0, \sigma^2_i)$$

where $\hat{y}_{ij}$ is the biomas or production for each taxa i at depth j (0,10…,50). Intercept of the model is given by $\beta_0 + a_{site}$ with changes randomly by a_{site}, and $b_i \times Depth_j$ represent the random variation of slope β_1.

Model validation was applied following (Zuur *et al.* 2009). Previous models were fitted using the restricted maximum likelihood estimation (REML) with functions *lmer* of the R package lme4 (Bates *et al.* 2016, R Core Team 2016). Finally, 5000 values from the posterior joint distribution of the model parameters were simulated with the function *sim* of the R package arm (Nievergelt *et al.* 2015). This function uses an analytical direct–simulation method with uninformative priors (Korner–Nievergelt *et al.* 2015). Obtained means of the simulated values from the joint posterior distribution of model parameters were used as estimates, and the 2.5% and 97.5% quintiles as lower and upper limits of 95% credible intervals. Finally, the marginal and conditional R^2 (as a technique to describe the predictive capacity of mixed effect model; Nakagawa & Schielzeth 2013) was calculated to assess model fit.

10 | References

Arnon, S. *et al.* Biophysicochemical process coupling controls nitrogen use by benthic biofilms. *Limnol. Oceanogr*. **52**, 1665–1671 (2007).

Aubeneau, A.F. *et al.* Effects of benthic and hyporheic reactive transport on breakthrough curves. *Freshw. Sci*. **34**, 301–315 (2015).

Baranov, V. *et al.* Effects of bioirrigation of non–biting midges (Diptera: Chironomidae) on lake sediment respiration. *Sci. Rep*. **6**: 27329 (2016).

Bardini, L. *et al.* Nutrient cycling in bedform induced hyporheic zones. *Geochim. Cosmochim. Acta.* **84**, 47–61 (2012).

Bates, D. *et al.* lme4: Linear Mixed–Effects Models using 'Eigen' and S4. R package version 1.1–12. (2016) https://cran.r–project.org/web/packages/lme4/index.html

Battin, T.J. *et al.* Contributions of microbial biofilms to ecosystem processes in stream mesocosms. Nature, **426**, 439–442 (2003).

Battin, T.J. *et al.* Biophysical controls on organic carbon fluxes in fluvial networks. *Nat. Geosci*. **1**, 95–100 (2008).

Battin, T. J. *et al.* The ecology and biogeochemistry of stream biofilms. *Nature Rev. Microbiol.* **14**, 251–263 (2016).

Bencala, K.E. A perspective on stream-catchment connections. *J. North Am. Benthological Soc*. **12**, 44–47 (1993).

Bencala, K.E. Hyporheic zone hydrological processes. *Hydrol. Process*. **14**, 2797–2798 (2000).

Bengtsson, G. Growth and metabolic flexibility in groundwater bacteria. *Microb. Ecol.* **18**, 235–248 (1989).

Benke, A.C. & Huryn, A.D. in *Methods in stream ecology* (eds Hauer, F.R. & Lambet, G.A) 691–710 (Academic Press, San Diego, 2007).

Bertics, V.J. & Ziebis, W. Biodiversity of benthic microbial communities in bioturbated coastal sediments is controlled by geochemical microniches. *ISME J.* **3**, 1269–1285 (2009).

Boano, F. *et al.* Hyporheic flow and transport processes: Mechanisms, models, and biogeochemical implications. *Rev. Geophys.* **52**, 603–679 (2014).

Boulton, A.J. Stream ecology and surface–hyporheic hydrologic exchange: implications, techniques and limitations. *Mar. Freshwater. Res*. **44**, 553–564 (1993).

Boulton, A.J. in *Stream and ground water* (eds Jones, J.B. & Mulholland, P. J.) 337–361 (Academic Press, San Diego, 2000).

Boulton, A. *et al.* Of spates and species: responses by interstitial water mites to simulated spates in a subtropical Australian river. *Exp. Appl. Acarol*. **34**, 149–169 (2002).

Boulton, A.J. *et al.* Ecology and management of the hyporheic zone: stream–groundwater interactions of running waters and their floodplains. *J. North Am. Benthological Soc*. **29**, 26–40 (2010).

Boyer, E. W. *et al.* Riverine nitrogen export from the continents to the coasts. *Biogeochem. Cycles*, **20**, GB1S91 (2006).

Briggs, M.A. *et al.* Practical limitations on the use of diurnal temperature signals to quantify groundwater upwelling. *J. Hydrol.* **519**, 1739–1751 (2014).

Buffington, J.M. & Tonina, D. Hyporheic exchange in mountain rivers II: Effects of channel morphology on mechanics, scales, and rates of exchange. *Geogr. Compass*. **3**, 1038–1062 (2009).

Calver, A. Riverbed permeabilities: Information from pooled data. *Groundwater*, **39**, 546–553 (2001).

Cardenas, M.B. The effect of river bend morphology on flow and timescales of surface water–groundwater exchange across pointbars. *J. Hydrol*. **362**, 134–141 (2008).

Carey, R.O. & Migliaccio, K.W. Contribution of wastewater treatment plant effluents to nutrient dynamics in aquatic systems: a review. *Environ. Manage.* **44**, 205–217 (2009).

Champion, P.D. & Tanner, C.C. Seasonality of macrophytes and interaction with flow in a New Zealand lowland stream. *Hydrobiologia* 441, 1–12 (2000).

Collier, K.J. *et al.* Trophic basis of production for a mayfly in a North Island, New Zealand, forest stream: contributions of benthic versus hyporheic habitats and implications for restoration. *N. Z. J. Mar. Freshwater Res.* **38**, 301–314 (2004).

Costanzo, S.D. *et al.* Ecosystem response to antibiotics entering the aquatic environment. *Mar. Pollut. Bull.* **51**, 218–223 (2005).

Cotton, J.A. *et al.* The effects of seasonal changes to in–stream vegetation cover on patterns of flow and accumulation of sediment. *Geomorphology* **77**, 320–334 (2006).

Cummins, K.W. *et al.* in *Stream ecology* (eds Barnes, J.R. & Minshall, G.W) 299–353 (Springer US, New York, 1983).

Dahm, C.N. *et al.* Nutrient dynamics at the interface between surface waters and groundwaters. *Freshw. Biol.* **40**, 427–451 (1998).

Danielopol, D.L. The distribution of the fauna in the interstitial habitats of riverine sediments of the Danube and the Piesting (Austria). *Int. J. Speleol.* **8**, 23–51 (1976).

Datry, T. *et al.* Intermittent rivers: a challenge for freshwater ecology. *BioScience*, bit027 (2014).

Davy–Bowker, J. *et al.* The distribution of benthic and hyporheic macroinvertebrates from the heads and tails of riffles. *Hydrobiologia*, **563**, 109–123 (2006).

Descloux, S. *et al.* Trait–based structure of invertebrates along a gradient of sediment colmation: Benthos versus hyporheos responses. *Sci. Total Environ.* **466**, 265–276 (2004).

Dodds, W.K. & Biggs, B.J. Water velocity attenuation by stream periphyton and macrophytes in relation to growth form and architecture. *J. North Am. Benthological Soc.* **21**, 2–15 (2002).

Dole–Olivier, M.J. The hyporheic refuge hypothesis reconsidered: a review of hydrological aspects. *Mar. Freshwater. Res.* **62**, 1281–1302 (2011).

Dole–Olivier M.J. *et al.* in *Groundwater ecology* (eds Danielopol D. *et al.*) 140–176 (Academic Press, New York, 1994).

Duff, J.H. & Triska, F.J. Nitogen biogeochemistry and surface–subsurface exchange in streams, in: Jones, J.B., Mulholland, P.J. (Eds.), *Streams and groundwaters*. Academic Press, San Diego, pp. 197–220 (2000).

Febria, C.M. *et al.* Bacterial community dynamics in the hyporheic zone of an intermittent stream. *ISME J.* **6**, 1078–1088 (2012).

Ferrington *et al.* Chironomidae, in *An introduction to the aquatic insects of North America* (eds Merritt, R.W. et. al.) 847–975 (Kendall/Hunt Publishing Company, Dubuque, 1996).

Findlay, S. & Sobczak. W.V. in *Streams and Ground waters* (eds Jones, J.B. & Mulholland, P.J.) 287–306 (Academic press, San Diego, 2000).

Fischer, H. A. *et al.* river's liver–microbial processes within the hyporheic zone of a large lowland river. *Biogeochemistry*, **76**, 349–371 (2005).

Foulquier, A., *et al.* Response of sediment biofilm to increased dissolved organic carbon supply in groundwater artificially recharged with stormwater. *J. Soils Sediments*, **11**, 382–393 (2011).

Gantzer, C.J. *et al.* Mass transport to streambed biofilms. *Water Res.* **22**, 709–722 (1988).

Gasith, A. & Resh, V.H. Streams in Mediterranean climate regions: abiotic influences and biotic responses to predictable seasonal events. *Annu. Rev. Ecol. Evol. Syst.* **30**, 51–81 (1999).

Gelman, A. *et al.* arm: data analysis using regression and multi–level / hierarchical models. R package, version 9.01 (2009). http://CRAN.R–project.org/package=arm.

Gerecht, K.E. *et al.* Dynamics of hyporheic flow and heat transport across a bed-to-bank continuum in a large regulated river. *Water Resour. Res.* **47**, W03524 (2011).

Gibert, J. *et al.* in *The Ecology and Management of Aquatic–Terrestrial Ecotones* (eds Naiman, R. J. & Décamps, H) 199–225 (UNESCO, Paris, 1990).

Gomez–Velez, J.D. *et al*. Effect of low‐permeability layers on spatial patterns of hyporheic exchange and groundwater upwelling. *Water Resour. Res.* **50**, 5196–5215 (2014).

Harvey, J.W. & Bencala, K.E. The effect of streambed topography on surface‐subsurface water exchange in mountain catchments. *Water Resour. Res*. **29**, 89–98 (1993).

Harvey, J.W. *et al.* Hyporheic zone denitrification: Controls on effective reaction depth and contribution to whole‐stream mass balance. *Water Resour. Res*. **49**, 6298–6316 (2013).

Hatch, C.E. *et al.* Quantifying surface water–groundwater interactions using time series analysis of streambed thermal records: Method development. *Water Resour. Res*. **42**, W10410 (2006).

Hendricks, S.P., 1993. Microbial ecology of the hyporheic zone: a perspective integrating hydrology and biology. *J. North Am. Benthological Soc.* **12**, 70–78.

Hendricks, S.P. & White, D.S. Physicochemical patterns within a hyporheic zone of a northern Michigan river, with comments on surface water patterns. *Can. J. Fish. Aquat. Sci.* **48**, 1645–1654 (1991).

Hernández Leal, L. *et al.* Occurrence of xenobiotics in gray water and removal in three biological treatment systems. *Environ. Sci. Technol*. **44**, 6835–6842 (2010).

Hirsch, R. *et al.* Occurrence of antibiotics in the aquatic environment. *Sci. Total Environ*. **225**, 109–118 (1999).

Hölker, F. *et al.* Tube‐dwelling invertebrates: tiny ecosystem engineers have large effects in lake ecosystems. *Ecol. Monogr.* **85**, 333–351 (2015).

Irvine, D.J. *et al.* Experimental evaluation of the applicability of phase, amplitude, and combined methods to determine water flux and thermal diffusivity from temperature time series using VFLUX 2. *J. Hydrol.* **531**, 728–737 (2015).

Irvine, D.J. & Lautz, L.K. High resolution mapping of hyporheic fluxes using streambed temperatures: Recommendations and limitations. *J. Hydrol*. **524**, 137–146 (2015).

James, A.B. *et al.* Do stream macroinvertebrates use instream refugia in response to severe short‐term flow reduction in New Zealand streams? *Freshwater Biol.* **53**, 1316–1334 (2008).

Jones Jr, J.B. & Holmes, R.M. Surface–subsurface interactions in stream ecosystems. *Trends Ecol. Evol*. **11**, 239–242 (1996).

Kalbus, E. *et al.* Measuring methods for groundwater–surface water interactions: a review. *Hydrol. Earth. Syst. Sc.***10**, 873–887 (2006).

Kasahara, T. *et al.* Treating causes not symptoms: restoration of surface–groundwater interactions in rivers. *Mar. Freshwater. Res.* **60**, 976–981 (2009).

Keery, J. *et al.* Temporal and spatial variability of groundwater–surface water fluxes: development and application of an analytical method using temperature time series. *J. Hydrol*. **336**, 1–16 (2007).

Köhler, H.R. & Triebskorn, R. Wildlife ecotoxicology of pesticides: can we track effects to the population level and beyond? *Science*, **341**, 759–765 (2013).

Korner–Nievergelt, F. *et al.* Bayesian data analysis in ecology using linear models with R, BUGS, and Stan. Academic Press, London (2015).

Krause, S. *et al.* Hyporheic hydrology: interactions at the groundwater‐surface water interface. *Hydrol. Process.* **23**, 2103–2107 (2009).

Langenhoff, A. *et al.* Microbial removal of the pharmaceutical compounds ibuprofen and diclofenac from wastewater. *Biomed. Res. Int*. **2013**, 1–9, (2013).

Lee, D.R.A device for measuring seepage flux in lakes and estuaries. *Limnol. Oceanogr.* **22**, 140–147 (1977).

Lewandowski, J. *et al.* Fate of organic micropollutants in the hyporheic zone of a eutrophic lowland stream: Results of a preliminary field study. *Sci. Total Environ*. **409**, 1824–1835 (2011).

Lewandowski, J. & Nützmann, G. Nutrient retention and release in a floodplain's aquifer and in the hyporheic zone of a lowland river. *Ecol. Eng.* **36**, 1156–1166 (2010).

Liu, Y. *et al.* Effect of Water Chemistry and Hydrodynamics on Nitrogen Transformation Activity and Microbial Community Functional Potential in Hyporheic Zone Sediment Columns. *Environ. Sci. Technol.* **51**, 4877–4886 (2017).

López–Rodríguez, M.J. *et al.* Diversity patterns and food web structure in a Mediterranean intermittent stream. *Int. Rev. Hydrobiol*. **97**, 485–496 (2012).

Luo, Y. *et al.* A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. *Sci. Total Environ.* **473**, 619–641.

Maazouzi, C. *et al.* Nutrient dynamics in riverbed sediments: effects of hydrological disturbances using experimental flow manipulations. *J. Soils Sediments*, **13**, 207–219 (2013).

Maazouzi, C. *et al.* Do benthic invertebrates use hyporheic refuges during streambed drying? A manipulative field experiment in nested hyporheic flowpaths. *Ecohydrology*, e1865 (2017).

Maddok, I.P. in *Groundwater and Geomorphology* (ed Brown, A. G.) 53–74 (Wiley, Chichester, 1995).

Malard, F. *et al.* Influence of groundwater upwelling on the distribution of the hyporheos in a headwater river flood plain. *Arch. Hydrobiol*. **157**, 89–116 (2003).

Marchant, R. Seasonal variation in the vertical distribution of hyporheic invertebrates in an Australian upland river. *Arch. Hydrobiol*. **134**, 441–457 (1995).

Maridet, L. & Philippe, M. Influence of substrate characteristics on the vertical distribution of stream macroinvertebrates in the hyporheic zone. *Folia Fac. Sci. Natur. Univ. Masaryk. Brun. Math*. **91**, 101–105. (1995).

Mermillod–Blondin, F. *et al.* Influence of macroinvertebrates on physico-chemical and microbial processes in hyporheic sediments. *Hydrol. Process*. **17**, 779–794 (2003).

McClain, M.E. *et al*. Biogeochemical hot spots and hot moments at the interface of terrestrial and aquatic ecosystems. *Ecosystems*, **6**, 301–312 (2003).

Miyake, Y. & Nakano, S. Effects of substratum stability on diversity of stream invertebrates during baseflow at two spatial scales. *Freshwater Biol.* **47**, 219–230 (2002).

Morad, M.R. *et al.* Quantification of pumping rate of Chironomus plumosus larvae in natural burrows. *Aquatic Ecol.* **44**, 143–153 (2010).

Mulholland, P.J. & DeAngelis, D.L. in *Streams and Ground waters* (eds Jones, J. B. & Mulholland, P.J) 149–168 (Academic Press, San Diego, 2000).

Mulholland, P.J. *et al.* Stream denitrification across biomes and its response to anthropogenic nitrate loading. *Nature*, **452**, 202–205 (2008).

Nakagawa, S. & Schielzeth, H. A general and simple method for obtaining R2 from generalized linear mixed‐effects models. *Methods Ecol. Evol.* **4**, 133–142 (2013).

Nogaro, G. *et al.* Ecosystem engineering at the sediment–water interface: bioturbation and consumer–substrate interaction. *Oecologia*, **161**, 125–138 (2009).

Olsen, D.A. & Townsend, C.R. Flood effects on invertebrates, sediments and particulate organic matter in the hyporheic zone of a gravel–bed stream. *Freshwater Biol.* **50**, 839–853 (2003).

Orghidan, T. Un nou domeniu de viata acvatica subterana:'biotopul hiporeic'. *Bull. Sti. sect. Biologie si sti. Agronau. si sect. Geologie si Geogr.* **7**, 657–676 (1955).

Pacioglu, O. & Robertson, A.L. The invertebrate community of the chalk stream hyporheic zone: spatio–temporal distribution patterns. *Knowl Manag Aquat Ecosyst*. **418**, 10 (2017).

R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria (2016). URL http://www.R–project.org/.

Reynolds Jr, S.K. & Benke, A.C. Chironomid production along a hyporheic gradient in contrasting stream types. *Freshw. Sci.* **3**, 167–181 (2012).

Robertson, A.L. Lotic meiofaunal community dynamics: colonisation, resilience and persistence in a spatially and temporally heterogeneous environment. *Freshwater Biol*. **44**, 135–147 (2000).

Robertson, A.L. & Wood, P.J. Ecology of the hyporheic zone: origins, current knowledge and future directions. *Fundam. Appl. Limnol./Arch. Hydrobiol.* **176**, 279–289 (2010).

Romaní, A.M. *et al.* Relevance of polymeric matrix enzymes during biofilm formation. *Microb. Ecol.* **56**, 427–436 (2008).

Roose–Amsaleg, C. & Laverman, A. M. Do antibiotics have environmental side–effects? Impact of synthetic antibiotics on biogeochemical processes. *Environ. Sci. Pollut. Res.* **23**, 4000–4012 (2016).

Rutherford, J.E. & Hynes, H.B.N. Dissolved organic carbon in streams and groundwater. *Hydrobiologia*, **154**, 33–48 (1987).

Sánchez–Pérez, J.M. *et al.* Role of the hyporheic heterotrophic biofilm on transformation and toxicity of pesticides. *Ann. Limnol. – Int. J. Lim*. **49**, 87–95 (2013).

Savant, S.A. *et al.* Convective transport within stable river sediments. *Water Resour. Res*. **23**, 1763–1768 (1987).

Schmid–Araya, J.M. Rotifers in interstitial sediments. *Hydrobiologia*, **387**, 231–240 (1998).

Schmid–Araya, J.M. Temporal and spatial distribution of benthic microfauna in sediments of a gravel streambed. *Limnol. Oceanogr*. **39**, 1813–1821 (1994).

Schulz, M. *et al.* Transformation of the X–ray contrast medium iopromide in soil and biological wastewater treatment. *Environ. Sci. Technol*. **42**, 7207–7217 (2008).

Shanafield, M. *et al.* Uncertainty in thermal time series analysis estimates of streambed water flux. *Water Resour. Res*. **47**, W03504 (2011).

Singer, G. *et al.* Microcosm design and evaluation to study stream microbial biofilms. *Limnol. Oceanogr. Methods*, **4**, 436–447 (2006).

Singer, G. *et al.* Physical heterogeneity increases biofilm resource use and its molecular diversity in stream mesocosms. *PLoS One*, **5**, e9988 (2010).

Smock, L.A. *et al.* macroinvertebrate production in three dimensions: channel surface, hyporheic, and floodplain environments. *Ecology*, **73**, 876–886 (1992).

Standley, E.H. & Boulton, A.J. Hydrology and the distribution of hyporheos: perspectives from a mesic river and a desert stream. *J. North Am. Benthological Soc.* **12**, 79–83 (1993).

Stead, T.K. *et al.* The contribution of subsurface invertebrates to benthic density and biomass in a gravel stream. *Arch. Hydrobiol.* **160**, 171–191 (2004).

Stegen, J.C. *et al.* Groundwater–surface water mixing shifts ecological assembly processes and stimulates organic carbon turnover. *Nat. Commun.* **7**, 11237 (2016).

Strayer, D.L. *et al.* Oxygen, organic matter, and sediment granulometry as controls on hyporheic animal communities. Arch. Hydrobiol. **140**, 131–144 (1997).

Stubbington, R. & Datry, T. The macroinvertebrate seedbank promotes community persistence in temporary rivers across climate zones. *Freshwater Biol.* **58**, 1202–1220 (2013).

Stubbington, R. *et al*. Low flow controls on benthic and hyporheic macroinvertebrate assemblages during supra-seasonal drought. *Hydrol. Process*. **23**, 2252–2263 (2009).

Tonina, D. & Buffington, J. M. Hyporheic exchange in mountain rivers I: Mechanics and environmental effects. *Geogr. Compass*, **3**, 1063–1086 (2009).

Townsend, C.R. *et al.* The intermediate disturbance hypothesis, refugia, and biodiversity in streams. *Limnol. Oceanogr*. **42**, 938–949 (1997).

Valett, H.M. *et al.* Perspectives on the hyporheic zone: integrating hydrology and biology. Introduction. *J. North Am. Benthological. Soc.* **12**, 40–43 (1993).

Vervier, P. *et al.* A perspective on the permeability of the surface freshwater–groundwater ecotone. *J. North Am. Benthological Soc.* **11**, 93–102 (1992).

Ward, J.V. The four–dimensional nature of lotic ecosystems. *J. North Am. Benthological Soc*. **8**, 2–8 (1989).

Ward, A.S. The evolution and state of interdisciplinary hyporheic research. *Wiley Interdiscip. Rev.* **3**, 83–103 (2016).

Ward, A.S. *et al.* Hydrologic and geomorphic controls on hyporheic exchange during base flow recession in a headwater mountain stream. *Water Resour. Res*. **48**, W04513 (2012).

White, D. S. Biological relationships to convective flow patterns within stream beds. *Hydrobiologia*, **196**, 149–158 (1990).

Williams, D.D. in *The Ecology of Aquatic Insects* (eds Resh, V. H. & Rosenberg) 430–455 (Praeger Publishers, New York, 1984).

Williams, D.D. & Hynes, H.B.N. The occurrence of benthos deep in the substratum of a stream. *Freshwater Biol.* **4**, 233–256 (1974).

Wondzell, S.M. The role of the hyporheic zone across stream networks. *Hydrol. Process*. **25**, 3525–3532 (2011).

Wondzell, S.M. Groundwater–surface–water interactions: perspectives on the development of the science over the last 20 years. *Freshw. Sci.* **34**, 368–376 (2015).

Wondzell, S.M. *et al.* An analysis of alternative conceptual models relating hyporheic exchange flow to diel fluctuations in discharge during baseflow recession. *Hydrol. Process.* **24**, 686–694 (2010).

Wright–Stow, A.E. *et al.* Hyporheic production is substantially greater than benthic production for a common New Zealand caddisfly. *Hydrobiologia*, **560**, 295–310 (2006).

Yang, S. & Carlson, K. Evolution of antibiotic occurrence in a river through pristine, urban and agricultural landscapes. *Water Res.* **37**, 4645–4656 (2003).

Zeglin, H.L. Stream microbial diversity responds to environmental changes: review and synthesis of existing research. *Front. Microbiol.* **6**, 454 (2015).

Zuur, A.F. *et al. Mixed Effects Models and Extensions in Ecology with R*. Springer, New York (2009).

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Review

Interplay of hydrology, community ecology and pollutant attenuation in the hyporheic zone

Ignacio Peralta-Maraver *, Julia Reiss, Anne L. Robertson

Department of Life Sciences, Roehampton University, London, UK

Chapter 3 | Environmental filtering and community delineation in the streambed ecotone

Ignacio Peralta-Maraver[1], Jason Galloway[2,5], Malte Posselt[3], Shai Arnon[4], Julia Reiss[1], Jörg Lewandowski[2,5] & Anne L. Robertson[1].

[1]Department of Life Sciences, Roehampton University, London, UK.
[2]Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Department Ecohydrology, Berlin, Germany.
[3]Department of Environmental Science and Analytical Chemistry, Stockholm University, Stockholm, Sweden.
[4]Zuckerberg Institute for Water Research, The Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Israel.
[5]Geography Department, Humboldt University, Berlin, Germany.
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1 | Abstract

A current controversy in ecology is whether biological communities are discrete biological entities or simply study units created for convenience; a debate that becomes even more heated when delimiting communities along ecotones. Here, we report an interdisciplinary study designed to address the interplay between environmental drivers and community ecology in a typical ecotone ecosystem: the streambed. Environmental filtering at a micro-scale determined how diversity, productivity and composition of the whole streambed assemblage varied with depth and with the direction of vertical water exchange. Biomass and production decreased with increasing depth, and were lower under upwelling than downwelling conditions. However, the rate at which biomass and production decreased with increasing depth differed significantly for different taxonomic groups. Using quantitative biocenosis analysis, we also showed that assemblages in close juxtaposition, such as benthic and hyporheic zone assemblages could be clearly distinguished as discrete communities with individual integrity. Vertical hydrodynamic conditions also influenced the demarcation between both communities; the benthic community reached greater depths in downwelling than in upwelling zones.

benthos | hyporheos | hyporheic exchange flow | environmental gradient | streams

2 | Introduction

Delineating communities has an august history reaching back to the writings of Theophrastus in the 4th century BC (Garnier *et al.* 2016). Even today the integrity of communities as real biological entities is disputed (Ricklefs *et al.* 2008, Brooker *et al.* 2009, Eichhorn 2016). This controversy stems from a common problem within ecology, which is that the community concept is a term frequently used but often only vaguely defined (Eichhorn 2016). Here, we accept a community as a set of biological populations inhabiting a certain habitat differing sufficiently (qualitatively and quantitatively) from other sets to be considered an ecological entity (Eichhorn 2016). While, we consider an assemblage as a group of organisms that are found together but where there is insufficient evidence to state that they form different communities (Eichhorn 2016). Delineating communities might be even harder in transitional ecosystems and at a local scale (Kraft *et al.* 2015), for example within a vertical gradient in streambed sediments (the surface–groundwater ecotone).

Traditionally the sediments of streams and rivers and their assemblages have been divided into two compartments according to their depth in the stream bed: the benthic zone (BZ), in direct contact with surface water and exposed to light and scouring forces of water, and the hyporheic zone (HZ), defined by shallow subsurface water pathways through river beds and banks beginning and ending at the river (Cardenas *et al*. 2016). This latter zone is also a biogeochemically active interface with a significant role in the functioning of aquatic ecosystems and in the retention and attenuation of nutrients and contaminants (Boulton *et al.* 2010). The line of demarcation between benthic (*benthos*) and hyporheic (*hyporheos*) assemblages can be recognised as the boundary between the benthic and hyporheic zones (the biological definition of the hyporheic zone; Orghidan 1955). However, distinguishing between the benthos and the hyporheos can be

challenging due to the dynamic and ecotonal nature of the HZ (Williams *et al.* 2010) and the question remains as to whether these two assemblages are real biological entities or merely units created by freshwater ecologists for convenience.

Depth below the surface and the direction (and magnitude) of the surface-groundwater exchange are acknowledged to be primary drivers of species distribution, assemblage structure and ecology in the streambed (Fraser & Williams 1998, Boulton *et al.* 1998, Dole-Oliver 1998, Sliva & Williams 2005, Miyake & Nakano 2006, Andrushchyshyn *et al.* 2007, Peralta-Maraver *et al.* 2018). At greater depths in the streambed sediment agglomeration occurs leading to reduced pore space and a reduction in oxygen availability, conditions that limit the vertical distribution of organisms with higher metabolic rates and larger sizes (Maridet & Philippe 1995, Strayer *et al*. 1997, Robertson & Wood 2010, Descloux *et al.* 2014). Thus, the ability of streambed organisms to colonise subsurface sediments depends on the biological traits that they possess (Nogaro *et al.* 2009, Robertson & Wood 2010, Descloux *et al.* 2014) and suggest that sediments will contain assemblages with relatively few and large invertebrates at the surface and that, with increasing depth, these will be replaced by a suite of numerous but small-bodied organisms (Schmid–Araya 1994, Stead et al. 2004). Water mixing in the HZ can lead to complex temporal and spatial flow patterns (Rutherford & Hynes 1987) in which downwelling (DW) and upwelling (UW) conditions may occur alternately (Boulton 1993) and the vertical extent of the HZ can be variable (Williams *et al.* 2010). The aforementioned vertical hydrodynamic patterns are reflected also in the biogeochemical conditions in the HZ. Typically, water downwelling from the surface contains higher levels of easily degradable organic matter and oxygen (Miyake & Nakano 2002, Davy–Bowker *et al.* 2006, Battin et al. 2016) and the abundance and diversity of streambed assemblages declines with increasing depth and is

higher in DW than in UW zones (Sliva & Williams 2005, Andrushchyshyn *et al.* 2007, Reynolds & Benke 2005). The selective pressures of the depth-dependent hydrodynamic and biogeochemical conditions can be considered micro-scale filters in streams and rivers (*sensu* Poff 1997) through which species must pass to constitute part of a given community (Poff 1997).

Most studies relating streambed assemblages with these environmental filters (depth and vertical hydrodynamic conditions) have focused on single taxonomic groups: Eumetazoa invertebrates (macroinvertebrates and meiofauna: multicellular organisms whose body size is greater 0.45 µm) (Sliva & Williams 2005, Reynolds & Benke 2005, Reynolds & Benke 2012) or Protozoa (eukaryotic single cell organisms) (Andrushchyshyn *et al.* 2007). Given that organisms differ in their ability to colonize the streambed sediments depending on their metabolic capabilities and body-size, we would expect a significant interaction between depth and taxonomic group (flagellates, ciliates, and multicellular invertebrates). Previous predictive regression models that explain the vertical gradient of biomass and secondary productivity in the streambed as responses of the depth gradient focus exclusively on large size organisms (mainly macroinvertebrates) and do not consider vertical hydrodynamic conditions. Thus, they explain only a small part of the observed variation highlighting the necessity of including more predictive variables and interactions (Peralta-Maraver *et al.* 2018).

To tease apart the nature and hierarchy of variables driving the structure and functioning of streambed systems, and to determine whether the benthos and hyporheos are indeed real biological entities, we took a challenging interdisciplinary approach. We combined techniques from hydrology and community ecology to determine flow in streambed sediments and to characterise the resident assemblages at the same spatial and temporal scales. We modelled the effect of vertical water flux and streambed depth

on the diversity, productivity and structure of streambed assemblages in a lowland river at a high spatial resolution. We included Eumetazoa invertebrates and two size-groups of protozoa (ciliates and flagellates) and thus, for the first time, our analysis spanned more than ten orders of magnitude in terms of body size. Comparing density across such a range of body sizes is problematic and so we focussed on comparisons of biomass, productivity and diversity.

Our overall aims were to demonstrate that the benthic zone and hyporheic zone are indeed different environments containing discrete communities that can be clearly delimited and that environmental filtering, resulting from the interplay of vertical hydrodynamic conditions and depth, rules the vertical gradient of biomass, production and diversity in streambed assemblages. We hypothesised that (1) the reduction in diversity, biomass and secondary productivity (responses) with increasing depth will depend on the body size of the taxonomic group. Accordingly, differential abilities to colonize the streambed sediments will result in an important interaction term of our predictive models. (2) Biomass, productivity and diversity are expected to be significantly higher under DW flow conditions, where there is higher dissolved organic carbon and oxygen, than under UW flow conditions. Thus, the magnitude and direction of vertical flow will be also an important predictor variable in our models. (3) With increasing depth, the benthic community will be replaced by a significantly different hyporheic community enabling the boundary between both communities to be delineated. (4) Vertical flow conditions (DW vs. UW) will determine the depth at which this boundary occurs; it will be deeper under DW conditions as a result of the downward influence of surface stream water.

3 | Methods

3.1 | *Study site*

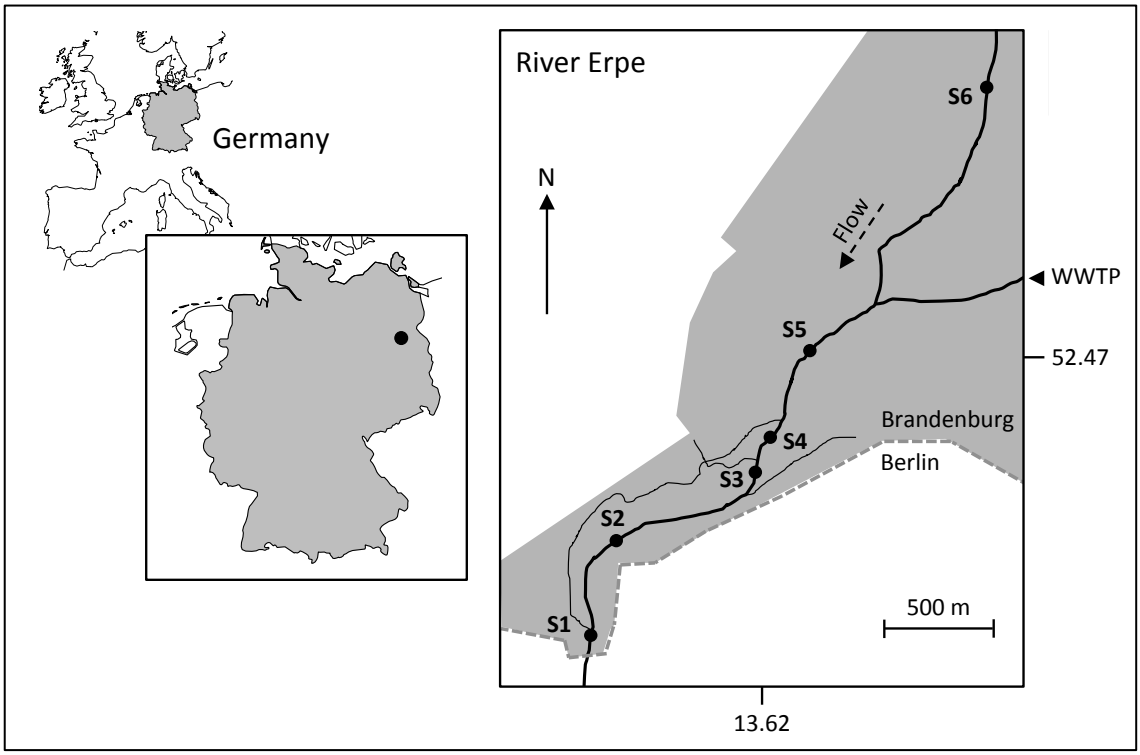

Fig 3.1. The study site at the River Erpe with sampling sites (S1 – S6). Effluent input from the municipal wastewater treatment plant (WWTP) Münchehofe is also shown. Grey shading in the right panel represents the protected area surrounding studied site.

The study was conducted on the lowland river Erpe, Northeast Germany (Fig 1), between 16th May and 16th June 2016. The catchment area is intensively affected by agriculture activities and the river receives daily treated wastewater releases from the Münchehofe waste water treatment plant (WWTP). As a result, streambed sediments are rich in organic carbon and nutrients (Gücker *et al.* 2006). Six sites were studied along a 3.5 km river stretch including one site upstream of the WWTP and five sites downstream (Fig 1). These locations were selected as potential UW or DW sites based on preliminary analysis of the hydraulic gradient between the river and groundwater table. At each site, a sediment portion of 1 m^2 area and 35 cm depth was selected in the streambed as a sampling point (Fig 3.2). Following Lewandowski and Nützmann (2010), two sediment cores (6 cm diameter) per sampling location were taken with a

modified Kajak corer (Fa. Uwitec). Cores were sliced in discrete 5-cm layers down to 35 cm and placed in plastic bags. Grain size distribution (GSD) was determined per layer after drying at 105 °C for 24 h. Percentage of GSD was classified by sequential sieving according to (Scheffer & Schachtsschable 1992). Additionally, water level data loggers (CTD-Diver, Westbay Instruments, Burnaby, Canada) were deployed in the water column at the west margin of every sampling point (Fig S3.1) providing water pressure data every 10 min (pressure accuracy ±0.5 cm H_2O). These data were used to provide elevation in cm relative to sea-level (m. a. s. l. = meter above sea level). These measurements were combined with cross-section profiling by measuring water depth every 10 cm along transects across the river (Fig 3.2).

3.2 | *Vertical hydrodynamics*

Vertical hydrodynamic conditions at different sites were characterised by coupling the location of the thermal extinction depth, measurement of the vertical streambed fluxes, and indirect analysis of redox conditions. For that purpose, lances with 8 temperature sensors (at 2.5, -2.5, -12.5, -17.5, -22.5, -27.5, -37.5 and -57.5 cm depth; resolution 0.04 °C; measurement frequency 10 min; UIT, Dresden, Germany) were installed vertically in the sediment (Fig S3.1). Thermal extinction depth (or specific penetration depth of a periodic surface temperature signal) was determined as the depth at which daily temperature variation is negligible (amplitude of daily temperature variation collapses).

Vertical streambed fluxes at each site were calculated through time series analysis of streambed thermal depth profiles during the whole study period (Hatch *et al.* 2006, Keery *et al.* 2007). The measured thermal time series were analysed with numerical one-dimensional advection-diffusion equations implemented in VFLUX 2 (MATLAB toolbox (Irvine *et al.* 2015, Gordon *et al.* 2012, Briggs *et al.* 2014). This software

calculates the one-dimensional vertical flux through saturated porous media based on Hatch *et al.* (2006) and Keery *et al.* (2007). Input parameters used here to compute the one-dimensional advection-diffusion equations are available as Table S3.1.

Finally, redox conditions in pore water through depth were characterised by profiling the concentration of aqueous ferrous iron (Fe^{2+}, highly reactive with oxygen) using one dialysis sampler (peeper) per study site. Peepers had seven chambers with a centre-to-centre separation of 5 cm (35 cm total length, vertical resolution of 5 cm). The capacity of each chamber was 20 ml. Detailed explanations of peeper preparation and set-up can be found in Hesslein (1976). Peepers were inserted vertically into the sediment to a depth of 30 cm with one chamber above the sediment and allowed to equilibrate with pore water for 21 days (Fig S3.1). After this period, they were removed from the sediment checking integrity of the dialysis membranes. One broken membrane was detected at site 5 in 10 cm depth and excluded from the analysis. Water from the rest of the chambers was collected and samples were put on ice until analysis, which took place within 12 hours of sample collection. Finally, ferrous iron (Fe^{2+}) concentration was measured photometrically using a segmented flow analyser (Skalar analytical B.V., EN ISO 11732 – Water quality).

3.3 | *Streambed community*

Sampling – The community of invertebrates and protists inhabiting the sediments was sampled using a modified Kajak corer. This corer has been shown to be very reliable and to provide representative samples of the streambed assemblages in sandy and silty habitats (Smock *et al.* 1992, Reynolds & Benke 2012, Majdi *et al.* 2017, Mathers *et al.* 2017). Three cores were taken per sampling point every seven days over 4 weeks. On each sampling date, the locations of the sampled cores were slightly altered to reduce disruptive effects on community assemblage (Fig S3.1). Discrete 5-cm layers were

sliced down to 35 cm, the lower limit of the community distribution. Equivalent layers from the three corers were then pooled (to maximise the representativeness of our samples) and well-mixed in plastic bags (giving 7 samples). From these plastic bags, subsamples for Protozoa processing (flagellates and ciliates) were transferred to 10 ml Sterilin plastic bottles and cooled until analysis. The remaining bag sample was then fixed with formalin at 4% containing Bengal rose stain.

Identification and quantification of organisms – In the laboratory, ciliates and flagellates were identified and counted alive within 24 h of sampling. Sub-samples were taken from the Sterilin bottles and processed under an Olympus BX50 microscope. Ciliate sub-samples were counted and identified using a Sedgewick rafter counting cell chamber (1 ml volume; Pyser-SGI limited, Edenbridge, United Kingdom), while flagellates were counted using a Neubauer cell counting chamber. Ciliates were identified to sub-class using identification keys (Foissner & Berger 1996), flagellates were treated as a single group. The remainder of the sample was rinsed through a 40-µm sieve to remove sediment and preserved again in formalin 4% containing Bengal-rose stain. Individuals were subsequently extracted under a Nikon SMZ-U stereomicroscope (30x), identified to the maximum possible resolution using identification keys (Rundle *et al.* 2002, Tachet *et al.* 2010) and counted. The length and width of all counted organisms (Protozoa and Eumetazoa invertebrates) were measured to the nearest micrometre.

Biomass, secondary production and diversity – Body dimensions of all counted organisms were converted to biovolume as described by Reiss & Schmid-Araya (2010). Then frequency of biovolume measurements was studied to verify the reliability of arranging Eumetazoa invertebrates, ciliates and flagellates as different size-groups. Following Putt & Stoecker (1996), protozoa individual biovolume was directly

transformed to carbon content assuming 0.14 pg C/μm^3, while for invertebrates it was first converted into fresh mass implementing published gravity values (Feller & Warwick 1998). Although this approach is widely used in the literature (i.e. Reiss & Schmid-Araya 2008, Tod & Schmid-Araya 2009), caution is needed because some taxa may give site-specific responses. Invertebrate individual carbon content was then calculated by using dry/wet mass ratio of 0.25 and dry mass/carbon content of 0.4 (Feller & Warwick 1998). Biomass (mg C/L) of all identified taxa was calculated for each sampling date and depth-layer by multiplying carbon content with individual density (ind/L).

To be consistent, the same non-cohort method was used to calculate secondary production of all taxonomic groups. Although this method is a suitable approximation to deal with the complex life histories of streambed assemblages and is widely used, it does not account for losses in production from factors such as migration, disease and predation (Benke & Huryn 2007). Total secondary production (P_t; mg C/L month) of identified taxa was calculated after Reiss and Schmid-Araya (2010) as the sum of interval production (P_i) between sampling dates (n = 3). Interval production was obtained as the product of mean biomass within the interval ($\hat{B}$), turnover rate of biomass per day (r) and interval duration in days (Δt, 7 days):

$$(1)\ P_i = \hat{B} \times r \times \Delta t$$

$$(2)\ P_t = \sum_{i=1}^{n} (P_i)$$

In the case of ciliates, flagellates, and permanent meiofauna (i.e. the non-insects), turnover rate of biomass was defined as the intrinsic rate of population increase. This rate was obtained by applying the allometric scaling relationship proposed by Reiss and Schmid-Araya (2010), which relates turnover rate of biomass with body mass of taxonomic groups. Subsequently, the critically temperature dependent intercept in

equation 1 was temperature-corrected per interval and along the depth gradient (using temperature data collected at the same time and scale with the thermal lances) by applying equations given by Gillooly *et al.* (2002). Turnover rate of biomass of meiofauna and macroinvertebrates was defined as daily growth rate (instantaneous growth method; Benke 1998, Benke & Huryn 2007). Daily growth rate values were obtained using published equations for different taxonomic groups (Reynolds & Benke 2005, Morin & Dumont 1994). Similarly, these values were also temperature-corrected per interval and along the depth gradient.

Finally, the diversity gradient along the depth per site was measured for every sampling date using the Shannon-Wiener´s diversity index (*H'*):

$$(3)\ H' = -\sum_{i=1}^{S} Pr_i \ln(Pr_i)$$

where S is the number of taxa in the community (here the assemblage at each depth-layer) and Pr_i the proportion of individuals in the community that belong to taxa *i* (Begon *et al.* 2005). This index is a useful method for following variability in relative density in a large number of taxa over time (Sager & Hasler 1969, Porter 1977).

3.4 | *Statistical analysis*

Biomass, secondary production and diversity – Two linear mixed effect models (LMMs) were applied to test the effect of vertical hydrodynamic conditions (factor with two levels: UW and DW), depth (continuous covariant) and taxonomic group (factor with three levels: Eumetazoa invertebrates, ciliates and flagellates) on the responses biomass and production respectively. Additionally, the effect of vertical hydrodynamic conditions and depth on Shannon-Wiener diversity was modelled using a Linear Mixed Model with Poisson distribution and natural logarithmic link function (Poisson-GLMM). In order to solve heterogeneity in the residuals, biomass and production variables were $\log_{10}$ transformed. Non-controlled variables (i.e. effect of WWTP input,

altered position of samples from one date to another) and temporal replication produced an intra-class correlation effect of the responses with the study site (a residual pattern with study site was observed during data exploration). Therefore, study site (*Site*) was incorporated in our models as a random factor (random intercept) in order to cope with this non-independence. Subsequently, a Widely Applicable Information Criterion (WAIC) was used to find the optimal models by combining main terms and interactions. Model validation was applied following Zuur et al. (2009) to verify the underlying assumptions, checking also the absence of overdispersion in the Poisson-GLMM (Person residuals / freedom degree = 0.04). Previous models were fitted using functions *lmer* and *glmer* of the R package lme4 (Bates et al. 2016, R Core Team 2016). Finally, 5000 values from the posterior joint distribution of the model parameters were simulated with the function *sim* of the R package arm (Gelman *et al.* 2009). This function uses an analytical direct-simulation method with uninformative priors (Korner-Nievergelt et al. 2015). Obtained means of the simulated values from the joint posterior distribution of model parameters were used as estimates, and the 2.5% and 97.5% quantiles as lower and upper limits of 95% credible intervals. Finally, the conditional-R^2 was calculated to assess model fit using the function *rsquaredGLMM* of the R package MuMIn (Nakagawa & Schielzeth 2013).

Delineation of communities– assemblage structure was assessed weekly at 5cm depth intervals (7 depths in total) through the sediment both in DW and UW zones in order to delineate the border between benthos and hyporheos. The Bray-Curtis similarity index was applied; this is a quantitative index that takes composition and proportional density of the organisms inhabiting each layer into account. An analysis of similarities (ANOSIM) was performed to test at which depth differences between upper layer and the subjacent sediment become statistically significant. Accordingly, significance of

ANOSIM statistic (*R*) was compared with its null distribution by permuting group membership 1000 times. Bray-Curtis index and ANOSIM analysis were performed using *vegdist* and *anosim* functions of the R-package vegan (Oksanen *et al.* 2016).

4 | **Results**

4.1 | *Study-site characterisation*

All study sites showed a relatively similar GSD distribution through the depth profile, aside from marginally higher proportions of interstitial clay at site 5 and sites 3, and higher proportions of gravels at sites 4 and 5 (Fig 3.2). The effect of the WWTP release was most evident on the river stage of downstream sites (1-5), which reached minima during the early morning and then rose sharply until noon remaining high until the evening (Fig 3.2). Despite the daily fluctuation in the surface water, it was possible to characterise sites 1, 3 and 5 as UW zones and sites 2, 4 and 6 as DW zones (Fig 3.3). The rate at which the daily amplitude of temperatures decreased was greater in sites 1, 3 and 5 than in sites 2, 4 and 6 (Fig 3.3). Therefore thermal extinction depth, and consequently surface water influence, did not penetrate so deep at sites 1, 3 and 5. These results agree with the obtained streambed vertical-flux values and redox conditions. Streambed vertical-flux values after VFLUX2 routines were similar using both the Hatch and the Kerry Amplitude method, thus, only the results from Hatch amplitude method are reported here. At sites 1, 3 and 5 the upward flux of water between temperature sensors (negative mean values, Fig 3.3) was dominant throughout the study period. In contrast, at sites 2, 4 and 6 downward flux of water into deeper layers (positive mean values, Fig 3.3) was observed. Finally, Fe^{2+} concentration in pore water (as an indirect measure of the redox zonation) increased much faster with increasing

depth in site 1, 3 and 5, while its concentration remained lower in the upper layers of site 2, 4, and 6 (Fig 3.3).

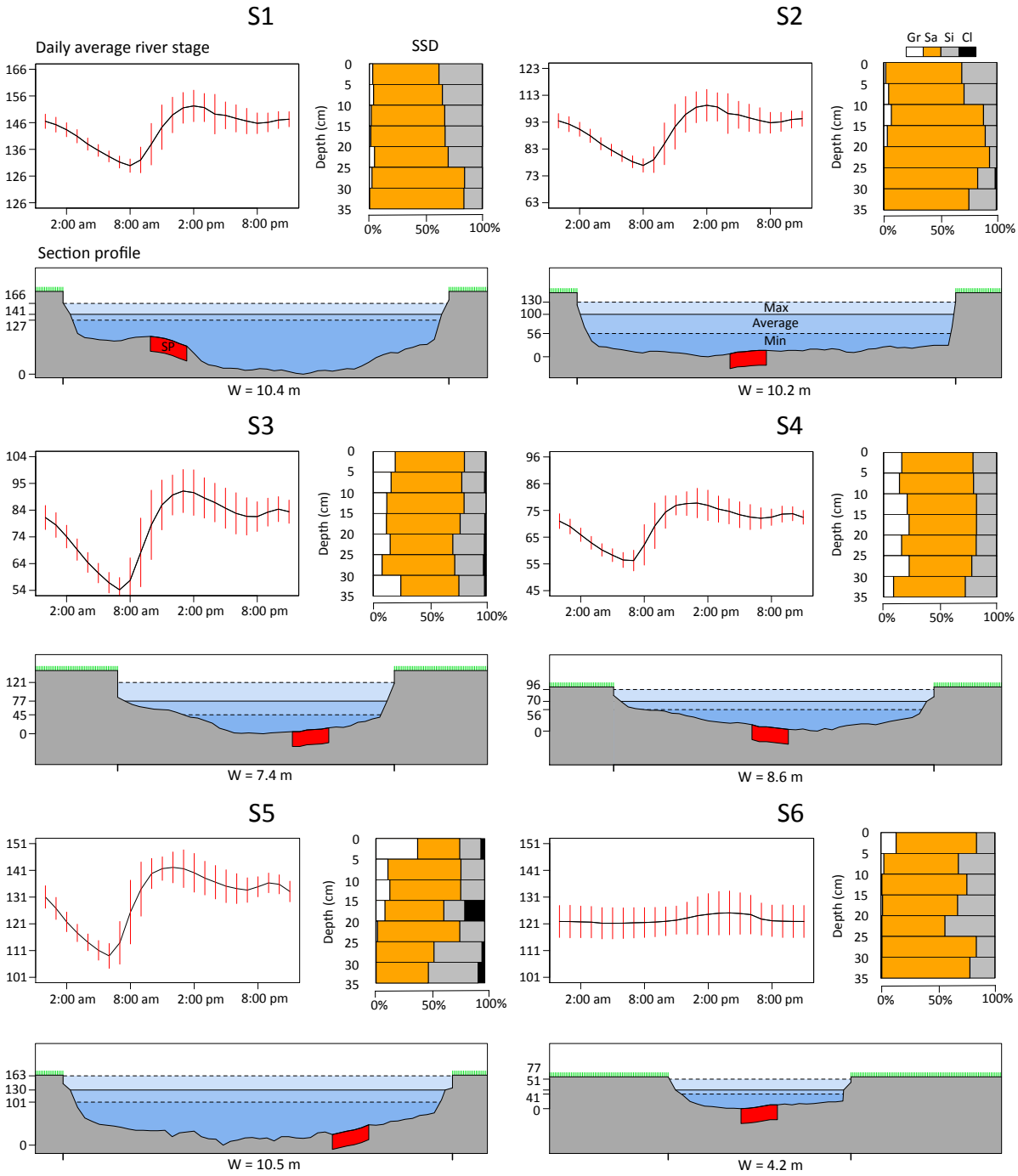

Fig 3.2. Hydrological and geomorphological features of each sampling site (S1 – S6). Each panel shows: (top-left) the daily average river stage (cm) of the period 16/05/2016 to 16/06/2016 with the mean value (black line) and the standard deviation of every hour (24 vertical red bars); (top-right) the proportion sediment size distribution (SSD: Gr = gravel, Sa = sand, Si = silt, Cl = Clay) for each 5 cm layer (between 0 and 35 cm); (bottom) the river cross section with average, maximum and minimum water stage during the above-mentioned study period. The location of sampled volume of sediments (SP) is also shown in red. W stands for width of the river.

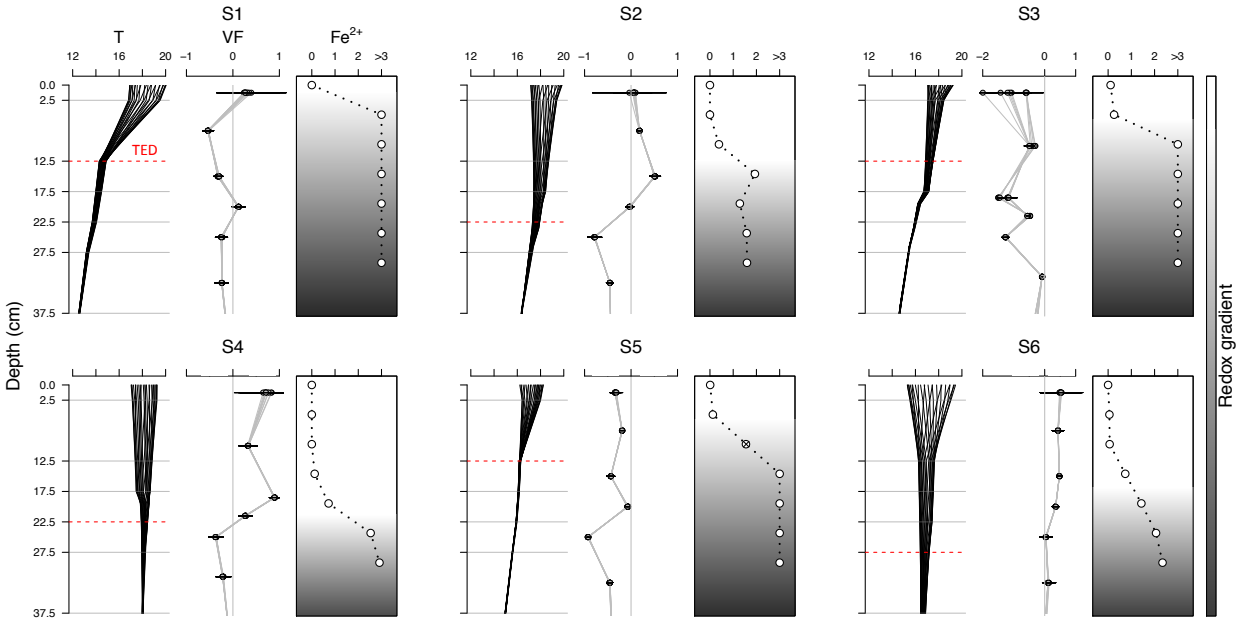

Fig 3.3. Daily amplitude of temperatures (T, °C), vertical flux profiles (VF, m/day) and ferrous iron concentration (Fe^{2+}, mg/L) at each sampling site. Temperature profiles consist of 24 lines for each hour of a diurnal cycle and are averaged based on 4 weeks of data (16/05/2016 to 16/06/2016). Thermal extinction depth (TED) is marked as a red dotted-line between the two temperature sensors in which the amplitude of daily temperature variation collapses. VF: The mean vertical flux between two neighbouring temperature sensors calculated with the 1-D numerical model VFLUX (Matlab) using the Hatch amplitude method[29]. Dots represent mean temperature values for every 2h during the day (12 dots per depth), while horizontal lines represent the standard deviations. Positive VF mean values indicate downward flux, negative values indicate an upward flux. Fe^{2+}: Vertical profile of ferrous iron concentrations based on one peeper deployment per sampling site (indirect measurement of the redox conditions). Note that the three methods agree that sites 1,3 and 5 are upwelling sites (DW), while sites 2,4 and 6 are downwelling sites (UW).

4.2 | *Biomass, secondary production, and diversity*

A total of 3874 Eumetazoa invertebrates, 2165 ciliates, and 420 flagellates were collected and identified to measure diversity, biomass and secondary production. The frequency of body sizes in the collected organisms clearly discriminates between the three studied groups (Fig S3.2). These measures were highly variable depending on taxonomic group, depth and vertical hydrodynamic conditions (Table S3.2). After WAIC routines, biomass and production models included vertical hydrodynamic conditions as a single factor and the interaction between depth and taxonomic group. In the case of the diversity model, depth and vertical hydrodynamic conditions were kept as effective parameters, while the interaction was not included. Fitted statistical models had a relatively high explanatory capability (Conditional $R^2 > 0.4$ in all cases, Fig 3.4a).

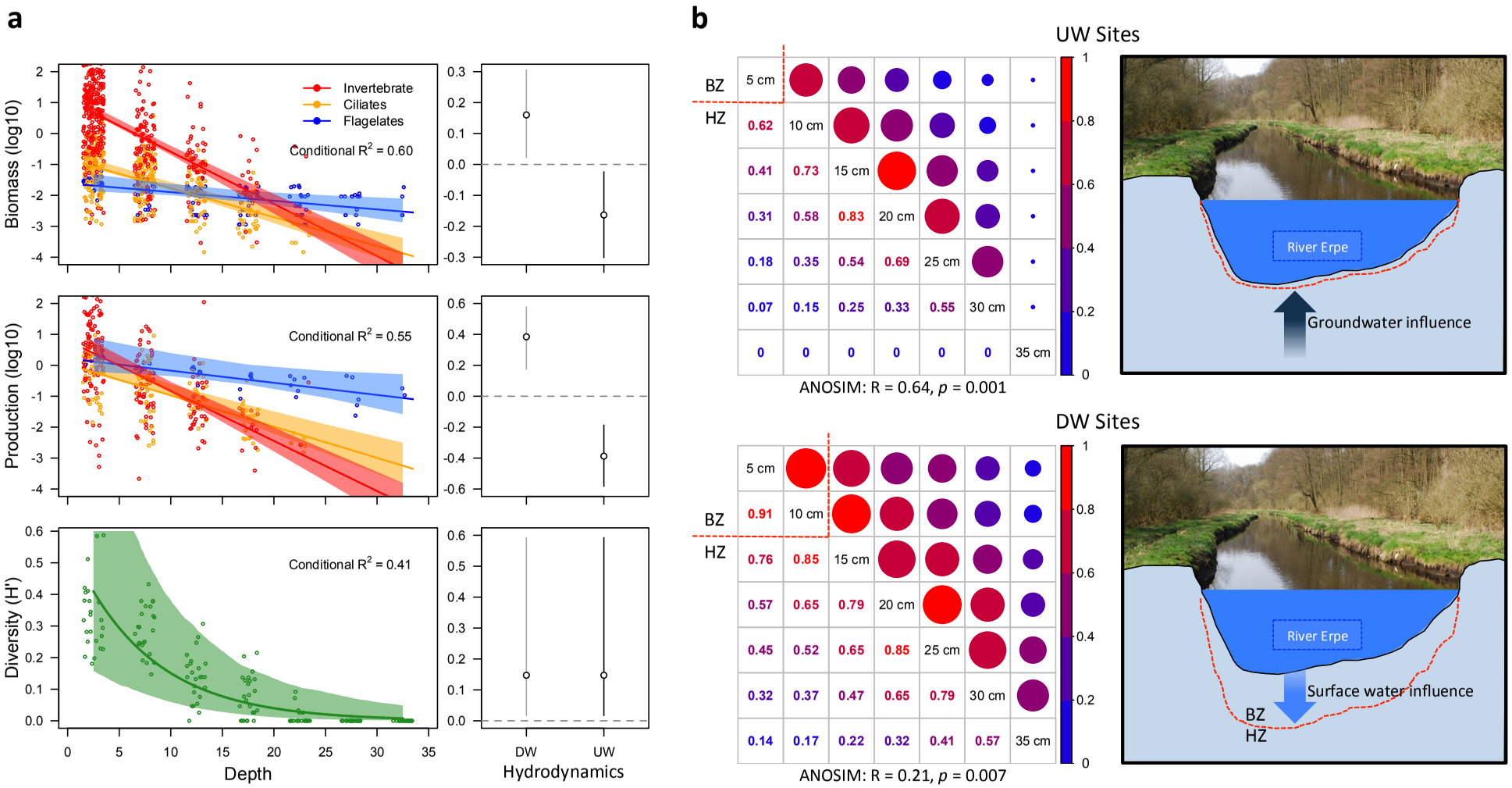

Fig 3.4. (**a**) Multiple linear regression models for biomass, production and Shannon-Wiener diversity. Coloured shaded area on the regression line and vertical bars represent the 95% credible intervals. (**b**) On the left, Bray-Curtis similarity matrix of the assemblage structure between depth layers in upwelling sites (UW) and downwelling sites (DW). Each matrix contains the numeric Bray-Curtis similarity value that resulted from comparing assemblage structure (composition and abundance) between sections. Similarity values range from maximum similarity (1.00) to maximum dissimilarity (0.00). A colour/size code is given to facilitate interpretation where big red circles represent maximum similarity, while small and blue circles represent maximum dissimilarity. The red dotted line marks the depth at which differences with the upper layers were significant for the ANOSIM analysis. This is represented schematically in the panels on the far right. Photographs in panel b were taken by Jörg Lewandowski (co-author of this study).

There was a significant, negative effect of depth on the responses: in the models, the depth coefficient (β_{depth}) was always significant in explaining biomass [β_{depth} = -0.09; 95% CrI = -0.11, -0.08; P ($\beta_{depth} < 0$) = 1], secondary production [β_{depth} = -0.13; 95% CrI = -0.11, -0.08; P ($\beta_{depth} < 0$) = 1] and diversity [β_{depth} = -0.13; 95% CrI = -0.20, -0.06; P ($\beta_{depth} < 0$) = 1] of Eumetazoa invertebrates, ciliates and flagellates (Fig 3.3a). Furthermore, the reduction in biomass and secondary production with increasing depth varied significantly depending on the taxonomic group (Fig 3.4a). Eumetazoa invertebrates showed the most abrupt reduction in biomass and secondary production with depth, followed by ciliates, and finally, flagellates. While Eumetazoa invertebrates dominate biomass from the surface to 20cm in depth, flagellates dominate total biomass below 20cm where Eumetazoa invertebrates are almost absent from the streambed and ciliate numbers are low (Fig4a).

The vertical flow conditions were also an important predictor variable in our models, at least for biomass and secondary production. Biomass and secondary production were significantly higher in DW sites than in UW sites (Fig 3.4a). In contrast, diversity values did not show any clear relationship with vertical hydrodynamic conditions (vertical hydrodynamics as the non-significant coefficient in Poisson-GLMM). Therefore, reduction in richness and proportion of taxa with increasing depth displayed a similar behaviour, independently of the vertical exchange of water [model equations, fitted coefficients, 95% CrI and probability (P) that the coefficient is different from 0 ($\beta \neq 0$) are available as Appendix 3.1 and Table S3.3].

4.3 | *Community structure*

We were able to detect the depth at which the benthic assemblage is replaced by the hyporheic assemblage in our system. After ANOSIM analysis, significant differences between surface and sub-surface assemblages were detected between 5 and

10 cm layers under UW conditions (only 62% of similarity), and between 10 and 15 cm layers under DW conditions (Fig 3.4b). Therefore, it was also possible to delineate the boundary between the BZ and the HZ purely based on biocenosis characteristics, and determine that the position of this boundary in the sediment differed depending on the direction of water flow. The ANOSIM model analysed whether the similarities of each depth layer between sites are smaller or equal to the similarities through depth. Our results showed that, at least for the limit between benthos and hyporheos (our main response), vertical differences in assemblage structure were significantly higher than horizontal differences (comparing between sites). We also found that the degree of change between the surface and deeper layers differed considerably depending on the vertical hydrodynamic conditions (the similarity analysis 1 – Bray-Curtis index; Fig 3.4b). Under UW conditions, community structure varied dramatically with depth often scoring 0 below 30 cm depth (studied organisms were not found in this environment, Table S3.2). In contrast, under DW conditions, community structure was more consistent and organisms colonized deeper layers of the streambed. Although our study design did not allow inferential comparative tests between sampling dates, we observed that the reported differences between assemblages at the surface and in deeper layers persisted throughout the study period for both vertical hydrodynamic treatments (Table S4), suggesting that the location of the boundary between assemblages was stable.

5 | Discussion

5.1 | *Hypothesis 1: the reduction in diversity, biomass and secondary productivity with increasing depth in the streambed will depend on the body size of the taxonomic group.*

The decline of biomass, secondary production and diversity of streambed populations with increasing depth has been widely reported in the literature and our

results support these findings (Smock *et al.* 1992, Schmid-Araya 1994, Dole-Oliver 1998, Storey & Williams 2004, Sliva & Williams 2005, Wright-Stow *et al.* 2006, Andrushchyshyn 2007, Reynolds & Benke 2012). However, no previous publications have quantified the relationship of these responses with depth in the streambed across a range of taxa with varying body sizes (Eumetazoa invertebrates, ciliates and flagellates). Our study clearly showed that the decline of biomass and secondary production was dependent on the taxonomic group; smaller body sizes penetrated deeper into the sediment. Schmid-Araya (1994) also found that the abundance of flagellates increased in comparison to larger ciliates at deeper layers in the streambed. Including the body size interaction term notably improved previous predictive models on depth–related biomass and secondary production of streambed assemblages (Peralta-Maraver *et al.* 2018) and highlighted the necessity of including small fauna, such as ciliates and flagellates, when determining the total production of streambed systems (Reiss & Schmid-Araya 2010).

It is intuitive that the larger body-size of Eumetazoa invertebrates constrains their ability to colonise agglomerated sediments (Maridet & Philippe 1995, Strayer et al. 1997, Descloux *et al*. 2014) and that the smaller size of ciliates and especially flagellates enables them to colonise the more compacted pore-space in deeper layers and dominate the assemblage in terms of biomass and secondary production. However, in our system there is also a strong redox gradient with depth, which may additionally constrain Eumetazoa invertebrates because they are highly dependent on oxygen availability in the streambed (Strayer *et al.* 1997). In contrast Protozoa include taxa known to support, and even prefer, anaerobic conditions (Foissner & Berger 1996, Andrushchyshyn *et al.* 2007,); unfortunately the level of protozoan taxonomic resolution in our study was insufficient to determine whether such groups were present

in the community. The environmental filtering concept has been traditionally applied to determine community identity at large spatial scales (such as altitudinal and latitudinal gradients; Blonder *et al.* 2015). Our findings suggest that micro-scale filters such as body size and metabolic requirements play a role in determining the community composition of streambed sediments and that environmental filtering is an important driver of gradients in the functional characteristics of organisms (i.e. Poff 1997, Hillebrand 2004). However, other factors that we did not measure (e.g. food sources, predation, competition) may also influence the observed vertical gradients in the community (Strayer 1994, Brunke & Gonser 1999).

5.2 | *Hypothesis 2: Community biomass, productivity and diversity will be significantly higher in DW flow conditions in comparison to UW flow conditions.*

Biomass and production also differed significantly depending on the vertical flow conditions, mostly supporting our second hypothesis. In DW zones surface water ingress resulted in markedly lower redox potential at deeper depths than in UW zones. This promoted the establishment of assemblages that had greater biomass and production than those in UW zones. This idea has been hypothesised previously (Schmid *et al.* 2000), however, our interdisciplinary approach to determining flow and the resident community in the streambed sediments enabled us, for the first time, to confirm the link between streambed flow paths and productivity of streambed assemblages. We demonstrated the role of DW sites as significant hot-spots of productivity, and therefore carbon processing in freshwater systems. We did not detect any effect of vertical hydrodynamic conditions on Shannon-Wiener diversity measurements, implying that there was a proportional simplification of the streambed assemblage through depth, independent of the vertical flux. Thus, similarly to Storey

and Williams (2016), depth was the strongest predictor of the assemblage diversity models. Diversity is very low at all the study sites in the Erpe River and this may be why we did not detect any differences across vertical hydrodynamic conditions despite the many reports of their critical importance in the literature (i.e. Storey & William 2004, Sliva & Williams 2005, Mathers *et al.* 2017).

5.3 | *Hypotheses 3 & 4: With increasing depth the benthic community will be replaced by a significantly different hyporheic community enabling the boundary between both communities to be delineated. Vertical flow conditions will determine the depth at which this boundary occurs.*

Using a fine scale approach based on biocenosis features we detected the depth at which the hyporheos replaced the benthos, and showed that these two communities, composing the whole streambed assemblage, are measurable ecological entities with individual integrity. Thus our third hypothesis was supported. Our findings provide definitive quantitative evidence for previous suggestions that the benthos is replaced by the hyporheos with increasing depth into the sediment (Storey & William 2004, Brunke, & Gonser 1999). Furthermore, in support of our final hypothesis, the line of demarcation between benthos and hyporheos was governed by surface and groundwater influence; in DW sites, benthos colonized deeper layers because the benthic biotope extended deeper into the substratum and this finding appeared to be persistent over the study period (Table S3.4) although slight variations in the similarity values (1- Bray-Curtis index) suggest that location of the boundary between communities might vary marginally.

6 | **Conclusions**

Our study confirms that the HZ is a spatially fluctuating ecotone between the surface stream and the deep groundwater (Fraser & Williams 1998) and that the hyporheos is a discrete and measurable biological community. It also draws attention to the importance of including precise measurements of vertical flux direction and magnitude when defining streambed system boundaries. Accurately defining natural system boundaries is an important aspect of designing ecological studies and for interpretation of results (Smock *et al.* 1992), but it is also central to defining ecosystems (Post *et al.* 2007). Our study also supports the often-mentioned necessity of multidisciplinary approaches in modern freshwater ecology. This strategy enabled us to assess the interplay of two central environmental micro-filters (depth gradient and vertical flow direction) in driving the productivity, diversity and organization of streambed assemblages.

7 | Acknowledgements chapter 3

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8 | Appendix chapter 3

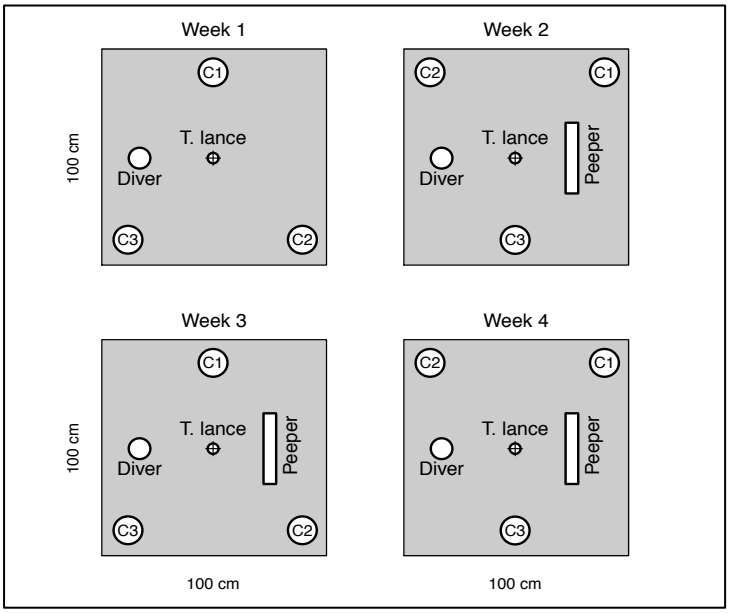


Fig S3.1. Scheme of the sampling design during time (from week 1 to week 4): Arial view of the 1 m^2 area (grey square) in which corers (C1, C2 and C3) were extracted and sampling devices [Water level data loggers (Diver), thermal lance (T.Lance) and Peeper] installed. Note that locations of the sampled cores were alternated weekly to avoid disrupting effects on community assemblage.

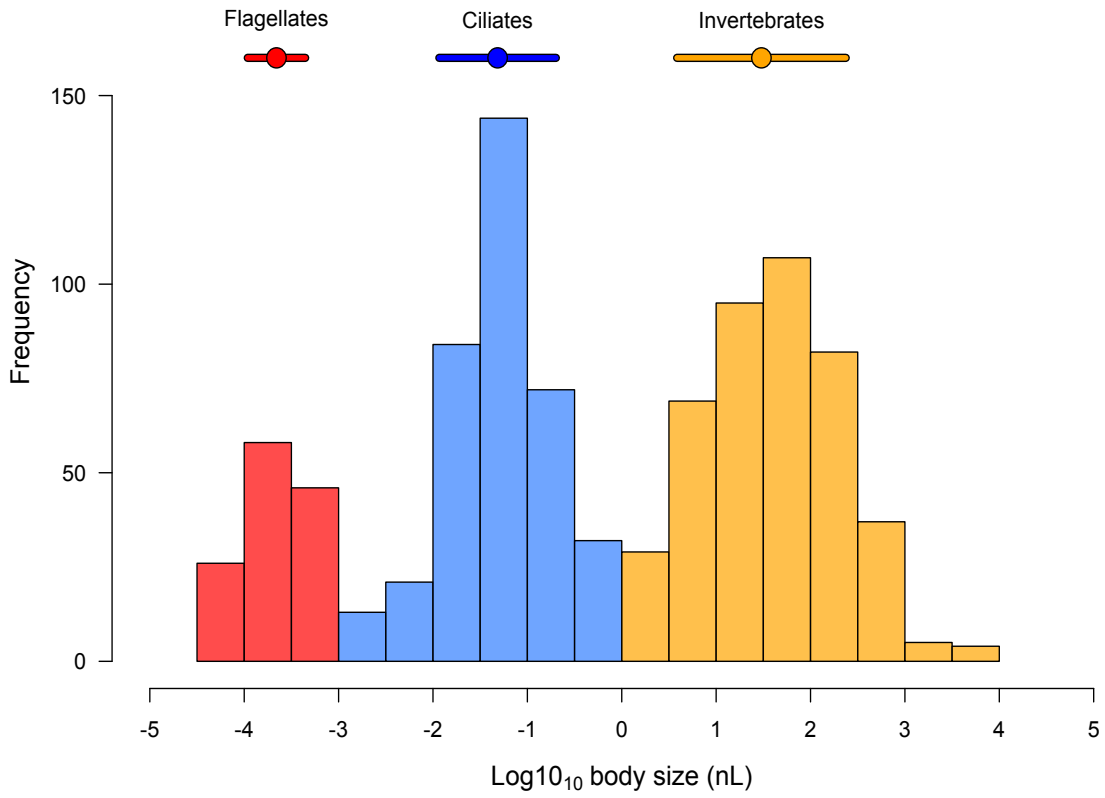


Fig S3.2. Frequency of body sizes ($\log_{10}$ biovolume, nL) in the streambed assemblage of the Erpe River. Dots represent mean values for flagellates (red), ciliates (blue) and Eumetazoa invertebrates (*Invertebrates*, orange). Horizontal lines represent the standard deviations. It was possible to discriminate three size groups, which clearly corresponded with the studied taxonomic groups.

Appendix 3.1.

Predictive models equations. (**1**) Linear Mixed Model in which responses $\hat{Y}$ (*Biomass* or *Production*) depend on the fixed intercept (β_0), random intercept (a_{site}), $Depth_i$ (continuous variables), $Group_j$ (factor with 3 levels: Invertebrates, ciliates and flagellates), interaction between $Depth_i$ and $Group_j$, and $Hydrodynamics_z$ (factor with 2 levels: UW and DW conditions). Both fixed residuals (ε_{ijz}) and random intercept follow a normal distribution. (**2**) Poisson Generalised Linear Mixed Model in which response (predicted mean of Shannon-Wiener diversity, η_{iz}) depend on the fixed intercept (β_0),

random intercept (a_{site}), $Depth_i$ (continuous variables) and $Hydrodynamics_z$ (factor with 2 levels: UW and DW conditions).

1) $$\mathbf{Log_{10}}(\hat{Y}_{ijz}) = \boldsymbol{\beta_0} + \mathbf{a}_{\text{site}} + \boldsymbol{\beta_1} \times Depth_i : \boldsymbol{\beta_2} \times Group_j + \boldsymbol{\beta_3} \times Hydrodynamics_z + \boldsymbol{\varepsilon_{ijz}}$$

$$\hat{Y}_{ijz} \sim \text{Norm}\ (0, \boldsymbol{\sigma^2_d})$$
$$\boldsymbol{\varepsilon_{ijz}} \sim \text{Norm}\ (0, \boldsymbol{\sigma^2})$$
$$\boldsymbol{a_{site}} \sim \text{Norm}\ (0, \boldsymbol{\sigma^2_{site}})$$

2) $$\boldsymbol{\eta_{iz}} = \boldsymbol{\beta_0} + \mathbf{a}_{\text{site}} + \boldsymbol{\beta_1} \times Depth_i + \boldsymbol{\beta_2} \times Hydrodynamics_z + \boldsymbol{\varepsilon_{ijz}}$$

$$Shannon_{iz} \sim \text{Pois}\ (\mu_{iz})$$
$$\text{E}(Shannon_{iz}) = \mu_{iz}$$
$$\mu_{iz} = \eta_{iz}$$
$$\boldsymbol{\varepsilon_{ijz}} \sim \text{Norm}\ (0, \boldsymbol{\sigma^2_d})$$
$$\boldsymbol{a_{site}} \sim \text{Norm}\ (0, \boldsymbol{\sigma^2_{site}})$$

Table S3.1: Inputs parameters used in the one-dimensional advection-diffusion equations during VFLUX2 routines.

Parameter	Value	Description	Notes
- ***rfactor***	12	- A positive integer factor by which to reduce the sampling rate.	- I used a *reduced sampling rate of 12 samples per fundamental cycle is recommended in order to eliminate spurious filtration artifacts.*
- ***windows***	1	- A positive integer determining the number of sensor-spacings. VFLUX2 will calculate flux between all the sensor pairs that are separated by the *window*.	- I had 8 sensors in the profile and *windows* =1. VFLUX2 calculated fluxes between sensors 1-2, 2-3, 3-4, and 4-5, 5-6, 6-7, and 7-8.
- ***Pf***	1	- Period of the fundamental temperature signal to filter and use for flux calculations (in days).	
- ***n***	0.28	- Total porosity of the sediment.	- Default value for sandy sediments.

Table continue

- ***beta***	0.001	- Dispersity (m).	- Default value for sandy sediments.
- ***Kcal***	0.0045	- Thermal conductivity, in cal/(s cm °C).	- Default value for sandy sediments.
- ***Cscal***	0.5	- Volumetric heat capacity of the sediment.	- Default value for sandy sediments.
- *Cwcal*	1.0	- Volumetric heat capacity of the water.	- Default value for sandy sediments.

Table S3.2. Mean and standard deviation (SD) values of biomass, production and Shannon-Wiener diversity index (Diversity) of different taxonomic groups, at upwelling sites (UW-sites) and down welling sites (DW), and at different depths.

Group	Depth	DW Sites		UW Sites	
		P (SD)	B (SD)	P (SD)	B (SD)
Invertebrates	5	37.844 (85.219)	103.350 (359.098)	20.052 (47.794)	52.720 (169.902)
	10	11.566 (52.662)	23.942 (124.416)	0.650 (1.324)	3.547 (17.787)
	15	5.760 (24.727)	2.197 (7.429)	0.091 (0.139)	0.230 (0.271)
	20	0.019 (0.016)	0.167 (0.280)	0.009 (NA)	0.046 (0.016)
	25	0.140 (0.195)	0.281 (0.141)	0.000	0.000
	30	0.000	0.000	0.000	0.000
	35	0.000	0.000	0.000	0.000
Ciliates	5	1.363 (1.049)	0.160 (0.270)	1.506 (2.139)	0.233 (0.355)
	10	0.282 (2.044)	0.130 (0.465)	0.515 (0.632)	0.073 (0.086)
	15	0.290 (0.347)	0.036 (0.057)	0.096 (0.079)	0.021 (0.036)
	20	0.064 (0.099)	0.008 (0.012)	0.015 (0.015)	0.004 (0.006)
	25	0.004 (0.002)	0.001 (0.001)	0.000	0.000
	30	0.000	0.000	0.000	0.000
	35	0.000	0.000	0.000	0.000
Flagellates	5	1.44 (0.356)	0.028 (0.006)	1.638 (0.525)	0.031 (0.015)
	10	0.968 (0.356)	0.020 (0.012)	0.651 (0.279)	0.015 (0.010)
	15	0.803 (0.068)	0.016 (0.010)	0.483 (0.225)	0.010 (0.008)
	20	0.438 (0.088)	0.009 (0.006)	0.238 (0.200)	0.006 (0.006)
	25	0.397 (0.051)	0.010 (0.007)	0.156 (0.067)	0.006 (0.006)
	30	0.297 (0.237)	0.007 (0.004)	0.065 (0.019)	0.007 (0.004)
	35	0.144 (0.051)	0.008 (0.008)	0.000	0.000
Diversity	5	0.360 (0.107)		0.310 (0.116)	
	10	0.240 (0.063)		0.325 (0.068)	
	15	0.140 (0.073)		0.137 (0.082)	
	20	0.100 (0.068)		0.057 (0.062)	
	25	0.023 (0.031)		0.000	
	30	0.000		0.000	
	35	0.000		0.000	

Table S3.3. Fitting models-coefficients and probability estimates. Mean, standard errors (SE), lower and upper credible intervals (2.5 and 97.5% CrI) and probability that the coefficient is different from 0 [P(β≠0)].

Log_{10}(*Biomass*)	**Mean**	**SE**	**2.5% CrI**	**97.5% CrI**	**P(β≠0)**
Intercept	-0.523	0.117	-0.757	-0.293	1.000 *
Depth	-0.094	0.009	-0.113	-0.076	1.000 *
Group (flagellate)	-0.911	0.206	-1.321	-0.511	1.000 *
Group (invertebrate)	2.126	0.143	1.854	2.416	1.000 *
Hydrology (UW)	-0.159	0.074	-0.295	-0.009	0.983 *
Depth x Group (flagellate)	0.064	0.013	0.038	0.090	1.000 *
Depth x Group (Invertebrate)	-0.078	0.014	-0.106	-0.052	1.000 *
Log_{10}(*Production*)					
Intercept	0.516	0.190	0.136	0.894	0.997 *
Depth	-0.105	0.014	-0.133	-0.076	1.000 *
Group (flagellate)	0.030	0.398	-0.752	0.794	0.530
Group (invertebrate)	0.898	0.231	0.459	1.349	1.000 *
Hydrology (UW)	-0.387	0.102	-0.587	-0.193	1.000 *
Depth x Group (flagellate)	0.063	0.023	0.018	0.106	0.996 *
Depth x Group (Invertebrate)	-0.065	0.020	-0.104	-0.025	1.000 *
Log_{10}(*Shannon*)					
Intercept	-0.221	0.743	-1.662	1.289	0.609
Depth	-0.135	0.033	-0.199	-0.067	1.000 *
Hydrology (UW)	-0.049	0.927	-1.811	1.821	0.561

Table S3.4. Bray-Curtis similarity matrix of the assemblage structure between depth layers in upwelling sites (UW) and downwelling sites (DW) every 7 days. Each matrix contains the numeric Bray-Curtis similarity value that resulted from comparing assemblage structure (composition and abundance) between sections every 7 days. Similarity values range from maximum similarity (1.00) to maximum dissimilarity (0.00).

		UW Sites							**DW Sites**						
		5cm	10cm	15cm	20cm	25cm	30cm	35cm	5cm	10cm	15cm	20cm	25cm	30cm	35cm
week 1	5cm	1,00							1,00						
	10cm	0,79	1,00						0,87	1,00					
	15cm	0,48	0,65	1,00					0,85	0,96	1,00				
	20cm	0,33	0,46	0,77	1,00				0,58	0,68	0,71	1,00			
	25cm	0,24	0,35	0,61	0,82	1,00			0,41	0,48	0,51	0,76	1,00		
	30cm	0,17	0,25	0,45	0,64	0,80	1,00		0,22	0,27	0,28	0,46	0,66	1,00	
	35cm	0,00	0,00	0,00	0,00	0,00	0,00	1,00	0,22	0,27	0,28	0,46	0,66	1,00	1,00

Continue table S3.4

week 2	5cm	1,00							1,00						
	10cm	0,58	1,00						0,86	1,00					
	15cm	0,31	0,61	1,00					0,65	0,76	1,00				
	20cm	0,29	0,57	0,94	1,00				0,43	0,52	0,73	1,00			
	25cm	0,23	0,47	0,82	0,87	1,00			0,41	0,49	0,70	0,97	1,00		
	30cm	0,00	0,00	0,00	0,00	0,01	1,00		0,29	0,35	0,52	0,76	0,79	1,00	
	35cm	0,00	0,00	0,00	0,00	0,01	0,92	1,00	0,00	0,00	0,00	0,00	0,00	0,01	1,00
week 3	5cm	1,00							1,00						
	10cm	0,55	1,00						0,81	1,00					
	15cm	0,39	0,79	1,00					0,70	0,88	1,00				
	20cm	0,26	0,56	0,74	1,00				0,42	0,56	0,66	1,00			
	25cm	0,00	0,00	0,00	0,01	1,00			0,26	0,36	0,44	0,72	1,00		
	30cm	0,00	0,00	0,00	0,01	0,91	1,00		0,18	0,26	0,31	0,54	0,80	1,00	
	35cm	0,00	0,00	0,00	0,01	0,83	0,92	1,00	0,00	0,00	0,00	0,00	0,01	0,02	1,00
week 4	5cm	1,00							1,00						
	10cm	0,80	1,00						0,89	1,00					
	15cm	0,31	0,43	1,00					0,60	0,68	1,00				
	20cm	0,11	0,15	0,38	1,00				0,57	0,65	0,96	1,00			
	25cm	0,00	0,00	0,00	0,00	1,00			0,44	0,51	0,80	0,84	1,00		
	30cm	0,00	0,00	0,00	0,00	1,00	1,00		0,35	0,41	0,67	0,70	0,86	1,00	
	35cm	0,00	0,00	0,00	0,00	1,00	1,00	1,00	0,19	0,23	0,40	0,42	0,54	0,67	1,00

9 | References

Andrushchyshyn, O.P. *et al.* Ciliate communities in shallow groundwater: seasonal and spatial characteristics. *Freshwater. Biol*. **52** 1745-1761 (2007).

Bates, D. *et al*. lme4: Linear Mixed-Effects Models using 'Eigen' and S4. R package version 1.1-12. (2016) https://cran.r-project.org/web/packages/lme4/index.html

Begon, M. *et al.* Ecology: From individuals to Ecosystems. 4th edition (Blakwell, Malden, USA, 2005).

Becker, R.A. & Wilks A.R. Maps. R package version 3.2.0 (2017). https://cran.r-project.org/web/packages/maps/maps.pdf

Benke, A.C. Production dynamics of riverine chironomids: extremely high biomass turnover rates of primary consumers. *Ecology* **79**, 899-910 (1998).

Benke, A.C., & Huryn, A.D. Secondary production of macroinvertebrates. In *Methods in Stream Ecology (Second Edition)* (pp. 691-710) (2007).

Battin, T.J. *et al.* The ecology and biogeochemistry of stream biofilms. *Nature Rev. Microbiol.* **14**, 251-263 (2016).

Blonder, B. *et al.* Linking environmental filtering and disequilibrium to biogeography with a community climate framework. *Ecology*, **96**, 972-985 (2015).

Boulton, A.J. Stream ecology and surface-hyporheic hydrologic exchange: implications, techniques and limitations. *Mar. Freshwater Res.* **44**, 553-564 (1993).

Boulton, A.J *et al.* The functional significance of the hyporheic zone in streams and rivers. *Annu. Rev. Ecol. Evol. Syst.* **29**, 59-81 (1998).

Boulton, A.J. *et al.* Ecology and management of the hyporheic zone: stream–groundwater interactions of running waters and their floodplains. *J. N. Am. Benthol. Soc*. **29**, 26–40 (2010).

Briggs, M.A. et al. Practical limitations on the use of diurnal temperature signals to quantify groundwater upwelling. *J. Hydrol*. **519**, 1739-1751 (2014).

Brooker, R.W. *et al.* Don't diss integration: a comment on Ricklefs's disintegrating communities. *Am. Na*t. **174**, 919-927 (2009).

Brunke, M., & Gonser, T. Hyporheic invertebrates: the clinal nature of interstitial communities structured by hydrological exchange and environmental gradients. *J. N. Amer. Benthol. Soc.* **18**, 344-362 (1999).

Cardenas, M.B. Hyporheic zone hydrologic science: A historical account of its emergence and a prospectus. *Water Resour. Res.* **51**, 3601-3616 (2015).

Davy–Bowker, J. *et al*. The distribution of benthic and hyporheic macroinvertebrates from the heads and tails of riffles. *Hydrobiologia* **563**, 109-123 (2006).

Descloux, S. *et al.* Trait-based structure of invertebrates along a gradient of sediment colmation: Benthos versus hyporheos responses. Sci. Total Environ. **466**, 265-276 (2014).

Dole-Oliver, M.J. Surface water-groundwater exchanges in three dimensions on the backwater of the Rhône River. *Freshwater. Biol*. **40**: 93-109 (1998).

Eichhorn, M. Natural systems: the organisation of life. (John Wiley and Sons, Chichester, 2016).

Feller, R.J. & Warwick, R.M. in Introduction to the study of meiofauna (eds Higgins, R. P. & Thiel, H.) 181—196 (Smithsonian Institution Press, Washington, 1998).

Foissner, W. & Berger, H.A user-friendly guide to the ciliates (Protozoa, Ciliophora) commonly used by hydrobiologists as bioindicators in rivers, lakes, and waste waters, with notes on their ecology. *Freshwater. Biol*. **35**, 375-482 (1996).

Fraser, B.G. & Williams, D.D. Seasonal boundary dynamics of a groundwater/surface-water ecotone. *Ecology* **79**: 2019-2031 (1998).

Garnier *et al*. *Plant Functional Diversity - Organism Traits, Community Structure, and Ecosystem Properties* (Oxford Univ. Press. London. 2016).

Gelman, A. et al. arm: data analysis using regression and multi–level / hierarchical models. R package, version 9.01 (2009). http://CRAN.R–project.org/package=arm.

Gordon, R.P. *et al.* Automated calculation of vertical pore-water flux from field temperature time series using the VFLUX method and computer program. *J. Hydrol.* **420**, 142-158 (2012).

Gillooly, J.F. *et al.* Effects of size and temperature on developmental time. *Nature* **417**, 70-73 (2002).

Gücker, B. *et al.* Effects of wastewater treatment plant discharge on ecosystem structure and function of lowland streams. *J. N. Am. Benthol. Soc.* **25**: 313-329 (2006).

Hatch, C.E. *et al.* Quantifying surface water–groundwater interactions using time series analysis of streambed thermal records: Method development. *Water Resour. Res.* **42**, W10410 (2006).

Hesslein, R.H. An in situ sampler for close interval pore water studies. *Limnol. Oceanogr.* **21**, 912-914 (1976).

Hillebrand, H. On the generality of the latitudinal diversity gradient. *Am. Nat.* **163**, 192-211 (2004).

Irvine, D. J. *et al.* Experimental evaluation of the applicability of phase, amplitude, and combined methods to determine water flux and thermal diffusivity from temperature time series using VFLUX 2. *J. Hydrol*. **531**, 728-737 (2015).

Keery, J. *et al*. Temporal and spatial variability of groundwater–surface water fluxes: development and application of an analytical method using temperature time series. *J. Hydrol.* **336**, 1-16 (2007).

Korner-Nievergelt, F. *et al*. Bayesian data analysis in ecology using linear models with R, BUGS, and Stan. (Academic Press. London, 2015).

Kraft, N.J. *et al*. Community assembly, coexistence and the environmental filtering metaphor. *Funct. Ecol.* **29**, 592-599 (2015).

Lewandowski, J. & Nützmann, G. Nutrient retention and release in a floodplain's aquifer and in the hyporheic zone of a lowland river. *Ecol. Eng.* **36**, 1156-1166 (2010).

Majdi, N. *et al.* It's the little things that count: Meiofaunal density and production in the sediment of two headwater streams. *Limnol. Oceanogr*. **62**: 151-163 (2017).

Maridet, L., & Philippe, M. Influence of substrate characteristics on the vertical distribution of stream macroinvertebrates in the hyporheic zone. *Folia Fac. Sci. Nat. Univ. Masaryk. Brun., Biol.* **91**, 101–105 (1995).

Mathers, K.L. *et al.* Benthic and hyporheic macroinvertebrate distribution within the heads and tails of riffles during baseflow conditions. *Hydrobiologia* **794**: 17-30 (2017).

Miyake, Y. & Nakano, S. Effects of substratum stability on diversity of stream invertebrates during baseflow at two spatial scales. *Freshwater. Biol*. **47**, 219–230 (2002).

Morin, A. & Dumont, P.A simple model to estimate growth rate of lotic insect larvae and its value for estimating population and community production. *J. N. Am. Benthol. Soc.* **13**, 357-367 (1994).

Nakagawa, S. & Schielzeth, H.A general and simple method for obtaining R2 from generalized linear mixed‐effects models. *Methods Ecol. Evol*. **4**, 133-142 (2013).

Nogaro, G. *et al*. Ecosystem engineering at the sediment–water interface: bioturbation and consumer–substrate interaction. *Oecologia* **161**, 125–138 (2009).

Oksanen, J. *et al.* Vegan: Community Ecology Package. R package version 2.4-1 (2016). https://cran.r-project.org/web/packages/vegan

Orghidan, T. Un nou domeniu de viata acvatica subterana:'biotopul hiporeic'. *Bull. Sti. sect. Biologie si sti. Agronau. si sect. Geologie si Geogr*. **7**: 657–676 (1955).

Peralta-Maraver, I. *et al.* Interplay of hydrology, community ecology and pollutant attenuation in the hyporheic zone. *Sci. Total Environ*. **610**, 267-275 (2018).

Poff, N.L. Landscape filters and species traits: towards mechanistic understanding and prediction in stream ecology. *J. N. Am. Benthol. Soc*. **16**, 391-409 (1997).

Porter, K.G. The Plant–Animal Interface in Freshwater Ecosystems: Microscopic grazers feed differentially on planktonic algae and can influence their community structure and succession in ways that are analogous to the effects of herbivores on terrestrial plant communities. *Am. Sci.* **65**, 159–170 (1977).

Putt, M. & Stoecker, D. An experimentally determined carbon:volume ratio for marine "oligotrichous" ciliates from estuarine coastal waters. *Limnol. Oceanogr.* **34**, 1097—1103 (1996).

R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria (2016). URL http://www.R–project.org/.

Reiss, J. & Schmid-Araya, J.M. Existing in plenty: abundance, biomass and diversity of ciliates and meiofauna in small streams. *Freshwater. Biol*. **53**, 652-668 (2008).

Reiss, J. & Schmid-Araya, J.M. Life history allometries and production of small fauna. *Ecology* **91**, 497-507 (2010).

Reynolds Jr, S.K. & Benke, A.C. Chironomid production along a hyporheic gradient in contrasting stream types. *Freshw. Sci*. **31**,167-168 (2012).

Reynolds, S.K. & Benke, A.C. Temperature-dependent growth rates of larval midges (Diptera: Chironomidae) from a southeastern US stream. *Hydrobiologia* **544**, 69-75 (2005).

Ricklefs, R.E. Disintegration of the ecological community: American Society of Naturalists Sewall Wright award winner address. *Am. Nat.* **172**, 741-750 (2008).

Robertson, A.L. & Wood, P.J. Ecology of the hyporheic zone: origins, current knowledge and future directions. *Fundam. Appl. Limnol./Arch. Hydrobiol*. **176**, 279–289 (2010).

Rundle, S.D. *et al.* Freshwater meiofauna: Biology and Ecology. (Blackhuys Publishers, Leiden, 2002).

Rutherford, J.E. & Hynes, H.B.N. Dissolved organic carbon in streams and groundwater. *Hydrobiologia,* **154**, 33-48 (1987).

Scheffer, F. & Schachtsschable, P. Lehrbuch del Bodenkunde, 13th edition (F. Enke, Frankfurt, 1992).

Sager, P.E. & Hasler A.D. Species diversity in lacustrine phytoplankton. I. The components of the index of diversity from Shannon's formula. *Am. Nat.* **103**, 51–59 (1969).

Schmid–Araya, J.M. Temporal and spatial distribution of benthic microfauna in sediments of a gravel streambed. *Limnol. Oceanogr*. **39**, 1813–1821 (1994).

Schmid, P.E. *et al*. Relation between population density and body size in stream communities. *Science*, **289**, 1557-1560 (2000).

Sliva, L. & Williams, D.D. Responses of hyporheic meiofauna to habitat manipulation. *Hydrobiologia* ***548***, 217-232 (2005).

Smock, L.A. *et al.* Lotic macroinvertebrate production in three dimensions: channel surface, hyporheic, and floodplain environments. *Ecology* **73**, 876–886 (1992).

Storey, R.G. & Williams, D.D. Spatial responses of hyporheic invertebrates to seasonal changes in environmental parameters. *Freshwater Biol.* **49**: 1468-1486 (2004).

Strayer, D.L. Limits to biological distributions in groundwater. In: J. Gibert, D. L. Danielopol, and J. A. Stanford (editors). *Groundwater ecology*. Academic Press, San Diego. pp. 287-310 (1994).

Strayer, D.L. *et al.* Oxygen, organic matter, and sediment granulometry as controls on hyporheic animal communities. *Arch. Hydrobiol.* **140**, 131–144 (1997).

Stead, T.K. *et al.* The contribution of subsurface invertebrates to benthic density and biomass in a gravel stream. *Arch. Hydrobiol*. **160**, 171–191 (2004).

Tachet, H. *et al*. 'Invertébrés d'eau douce. Systématique, biologie, écologie.' (Centre National de la Recherche Scientifique editions, Paris, 2010).

Tillman, D.C. *et al.* Subsurface hydrology and degree of burial affect mass loss and invertebrate colonisation of leaves in a woodland stream. *Freshwater Biol***. 48**, 98-107 (2003).

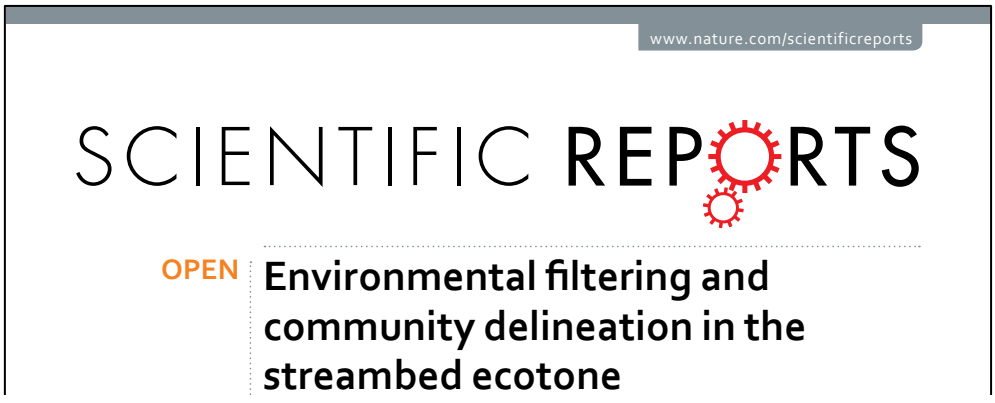
Tod, S.P., & Schmid-Araya, J.M.. Meiofauna versus macrofauna: secondary production of invertebrates in a lowland chalk stream. *Limnol. Oceanogr*, **54**: 450-456 (2009)

Post, D.M. *et al.* The problem of boundaries in defining ecosystems: a potential landmine for uniting geomorphology and ecology. *Geomorphology* **89**, 111-126 (2007).

Williams, D.D. *et al.* Ecotonal and other properties of the hyporheic zone. Fundam. Appl. Limnol./Arch. Hydrobiol. **176**, 349-364 (2010).

Wright-Stow, A.E. *et al.* Hyporheic production is substantially greater than benthic production for a common New Zealand caddisfly. *Hydrobiologia*, 560, 295-310 (2006).

Zuur, A.F. *et al.* Mixed Effects Models and Extensions in Ecology with R. (Springer, New York, 2009).



Chapter 4 | Nutrients and predators control the biodegradation of emerging organic contaminants by bacteria

Ignacio Peralta–Maraver*[1], Julia Reiss[1], Cyrus Rutere[2], Marcus A. Horn[2,3] & Anne L. Robertson[1]

[1]Department of Life Sciences, Roehampton University, London, UK

[2]Department of Ecological Microbiology (EMIC), University of Bayreuth, Bayreuth, Germany

[3]Institute of Microbiology, Leibniz Universität Hannover, Hannover, Germany.

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1 | Abstract

1. Water is considered the most indispensable natural resource, yet organic pollution of freshwater sources is widespread. In recent years, there has been increasing concern over the vast array of emerging organic contaminants (EOCs) in the effluent of wastewater treatment plants (WWTPs) that end in natural freshwater systems. Bacterial assemblages inhabiting the pore–space of streambed sediments may degrade several of these EOCs.
2. Here, we report how dissolved organic carbon (glucose), cell density of pelagic bacteria degraders and predator–prey interactions drive the capacity of streambed sediments to process a model EOC (ibuprofen). Glucose had a significant positive effect on bacterial densities. Meanwhile, the effect of predator presence followed a hormesis–like effect, in which low and medium levels of predator density stimulated bacterial population growth. The resulting increase in cell densities of the bacterial degrader produced a higher consumption of ibuprofen.
3. The hormesis–like effect of predation density interacted synergistically with glucose availability and degrader cell density, resulting in an intensification of the responses. Thus, low and medium levels of predator density not only boost bacterial population growth but also its efficiency in processing ibuprofen.
4. Our results suggest (although we did not directly determine the relationship between glucose and ibuprofen removal) that where nutrients are plentiful densities of bacterial degraders increase and, consequently the self–purifying capability of the system rises. Our findings enhance our understanding of the mechanisms by which streambed assemblages drive the processing of EOCs. Furthermore, our results emphasise the importance of preserving natural predator–prey interactions in order to maintain and sustain ecosystem services.

microcosms experiment | bioreactor | micropollutants | contaminants processing | hyporheic zone | trade–off | predator–prey dynamics | microbial food web

2 | Introduction

The majority of the world's rivers contain high levels of organic contaminants derived from anthropogenic activities (Mulholland *et al.* 2008, Vörösmarty *et al.* 2010, Wen *et al.* 2017) and the resulting pollution of freshwater sources has contributed to local and regional losses of biodiversity and a reduction in ecosystem services such as clean drinking water (Malaj *et al.* 2014), leading to global public concern (Vörösmarty *et al.* 2010, Schwarzenbach *et al.* 2006). These problems are expected to become more acute in the coming decades as water scarcity increases, even in regions currently considered water–rich (Vörösmarty *et al.* 2010, Wen *et al.* 2017, Shannon *et al.* 2008). In addition, conventional wastewater treatment plants WWTPs are remarkably inefficient at removing emerging organic contaminants (EOCs) (Evgenidou *et al*. 2015, Verlicchi *et al.* 2012), resulting in widespread and continuous pollution that has the potential to affect all levels of biological organization (Stamm *et al.* 2016). These EOCs are compounds of anthropogenic origin that have trace concentrations in natural systems (ng–mg per litre) but disproportionally high biological activity (Stamm *et al.* 2016) and include thousands of daily–use synthetic chemicals, such as pharmaceuticals and personal care products (Pal *et al.* 2010). Ibuprofen is one such example, it is the most consumed non–steroidal anti–inflammatory drug worldwide (Heckmann *et al.* 2007) and its constant release into freshwater systems worldwide has potential toxic and hazardous effects both on aquatic communities and human health (Pal *et al.* 2010).

Most WWTPs effluents are discharged to surface streams and rivers where water is exchanged between the open channel and the saturated permeable streambed sediments (called the hyporheic zone: HZ) (Findlay 1995). The large volume of pore–space in the HZ is the biotope of many microscopic organisms, such as bacteria and eukaryotic single–cell organisms (Findlay 2010, Battin *et al.* 2016). Bacteria may live

attached to the pore–space surface embedded in a polysaccharide matrix (biofilms) (*see* Battin *et al.* 2016), but may also live suspended in the interstitial pore water (i.e. free–living bacteria or detached from the biofilms) (Harvey & Garabedian 1991, Fuller *et al.* 2000, Febria *et al* 2012). The diverse bacterial consortia in these pore–spaces are key sites of enzymatic activity with the ability to biodegrade dissolved substances in the pore water (Findlay 2010, Flemming & Wingender 2010) including EOCs (Lewandowski *et al.* 2011, Stegen *et al.* 2016), in some cases even more efficiently than WWTPs (i.e. Schulz *et al.* 2008, Radke *et al* 2009). Moreover, the high residence time of water and solutes in the hyporheic zone enhances biochemical reactions mediated by the bacterial assemblages (Lewandowski *et al.* 2011, Bardini *et al.* 2012). These bacterial assemblages depend on the input of nutrients dissolved in surface water (Bengtsson 1989); higher inputs of dissolved organic carbon (DOC) result in greater bacterial biomass and activity (Foulquier *et al.* 2011). Most of the research into dissolved solute uptake by streambed bacteria has focussed on biofilms, while pelagic bacteria have received much less attention. Biofilm theory holds that uptake of solutes is diffusion–limited by the thickness of the biofilm polysaccharide matrix (Gantzer *et al.* 1988). Before uptake, solutes must pass initially from the pore water to the biofilm surface (external mass transfer) and then through the polysaccharide matrix to the cells (internal mass transfer) (Battin *et al.* 2003). In contrast, pelagic bacteria are much more exposed to dissolved solutes, and therefore they are likely to have an important role in the biodegradation processes. At the same time the life activities of eukaryotic single–cell organisms (swimming in the pore–space and grazing on biofilms), promote water mixing and boost bacterial activity (*sensu* Peralta-Maraver *et al.* 2018). This interface system is thus a water–purifying bioreactor (hyporheic bioreactor) but the mechanisms driving its processing of EOCs are unknown, in part because of the complexity of the

system (Peralta-Maraver *et al.* 2018). However, this knowledge is essential for the development of successful and long–term EOC mitigation strategies (Schwarzenbach *et al.* 2006).

We designed a controlled microcosm experiment reducing the complexity inherent in natural systems to elucidate the mechanisms behind EOCs processing by the hyporheic bioreactor, simulating idealised pore–space conditions in the HZ after a daily release of water from a WWTP. We explored how nutrient availability and predation presence influences pelagic bacteria abundance and, by extension, their capacity to remove EOCs. We incubated an environmental strain of bacteria (isolated from a real system) with the ability to consume ibuprofen (as model EOC) in the presence of different densities of the microscopic bacteria predator *Tetrahymena pyriformis* (ciliate species as model predator), under different levels of glucose availability (simple carbon source as model nutrient) and at a standardised initial concentration of dissolved ibuprofen. First, we focussed on the response of the pore–space bacterial population to bioavailable DOC and the presence and abundance of the bacteria predator. Second, we investigated how the increase in cell density of the pelagic bacterial degrader and its interaction with glucose availability (also as a substrate competitor with ibuprofen) and density of predators determined the EOC removal. We hypothesise that both increase in glucose and predation promote pelagic bacteria metabolism and, consequently ibuprofen uptake. Finally, we used these results to develop a conceptual overview of EOCs degradation in the streambed. Our results illuminate the functioning of an important ecosystem service and open the door to a new line of research on the fate of EOCs in freshwater systems.

3 | Methods

3.1 | *Organisms*

We designed a model predator–prey system with a non–pathogenic, ibuprofen [2–(4–isobutylphenyl–propionic acid)] degrading environmental bacterial strain as prey and the axenically cultured ciliated protozoon *Tetrahymena pyriformis* CCAA 1630/1 W (Culture Collection of Algae and Protozoa, SAMS Limited, Scottish Marine Institute, Scotland, UK) as model predator. The environmental bacterial strain was isolated from the hyporheic sediment of a natural stream system (see Supplementary methods for detailed description of the bacteria strain isolation and preparation). The protozoan *T. pyriformis* (Fig 4.3) is a ubiquitous ciliate commonly used as a model organism in experimental research with a formidable growth rate, carrying capacity and swim–velocity (Altermatt *et al.* 2015). Importantly this protozoan can be cultured in the absence of bacteria (axenic) but will prey on bacteria when they are available.

Emerging organic contaminant– ibuprofen was selected as model EOC because it is one of the most widely used non–steroidal anti–inflammatory drugs (Heckmann *et al.* 2007) and it has been detected in bodies of water worldwide (Han *et al.* 2010). Furthermore, ibuprofen attenuation in the pore–space of the HZ has been reported (Lewandowski, J. *et al.* 2011).

3.2 | *Stock cultures*

Stocks of environmental bacteria were cultured in ibu–growth medium (sugarless–growth medium with 0.08 mg/mL ibuprofen, Table S4.1) at 15 °C and pH 7 for 4 weeks before experimental activities. Fresh stocks of environmental bacteria were prepared every 48 hours by transferring 2.0 mL from old stock into a 500 mL glass bottle with axenic ibu–growth medium. *T. pyriformis* was cultured in 2% protease peptone medium at 15 °C and pH 7. This medium is especially well suited to grow *Tetrahymena* sp. under axenic conditions (Cassidy–Hanley 2012). A fresh stock with a carrying capacity

of ciliate population density was prepared before the experimental procedures. For this purpose, an aliquot (~ 5mL) from a mature ciliate stock was transferred to culture bottles with fresh 2% protease peptone medium. Then, 5 sub–samples were taken from these fresh cultures every 12h in which ciliates were counted using a Sedgewick Rafter counting cell chamber (1 ml volume; Pyser–SGI limited, Edenbridge, United Kingdom). An asymptotic growth in ciliate population was reached after 7 days [Mean (SD) = 25.62 (2.09) × 10^4 ind/mL, Fig S4.1]. Bacteria and ciliate cultures were kept, and experimental procedures were done, in thermostatically controlled incubators (Lovibond, Amesbury, UK). Contamination and pH were checked daily during the pre–experiment activities. All culture and experimental procedures were carried out under axenic conditions using a class II microbiological safety cabinet (Envair ltd. Lancashire, UK).

3.3 | *Experimental design*

We used a two–way factorial design with different levels of glucose availability and ciliate density as the two main factors (five levels of each). Final density of pelagic bacteria and ibuprofen final concentration were the responses. Different levels of glucose availability were obtained following a serial dilution: 0.00 (control), 0.08 (poor), 0.31 (medium), 1.25 (rich) and 5.00 (very rich) mg/mL glucose in 2 mL final volume. Ciliates were first harvested by centrifugation from a fresh stock reaching its carrying capacity. Then, different levels of ciliate density were obtained by following a serial dilution: 0.00 (control), ~0.41 (low), ~1.62 (medium), ~ 6.50 (high), ~ 26.00 × 10^4 (very high) ind/mL in 2 mL final volume. Initial ibuprofen concentration was maintained at 0.08mg/ml in all the treatments. This design was fully crossed and balanced by combining both factors in 25 different treatments and replicated 5 times each (125 microcosms). The microcosms were individual wells in 96 multi–well tissue

culture plates (125 wells, each 1.5 cm diameter and 2 mL volume). Two 96 multi–well plates were used, in which replicates of microcosms with the different treatments were randomly located. Microcosms were prepared following a sequenced protocol and incubated for 24h. After this period, pelagic bacteria counts and final ibuprofen concentration were measured using an Accuri C6 flow cytometer (BD Biosciences) and a liquid chromatography–targeted tandem mass spectrometer (LC–MS/MS/MS). A detailed explanation of the microcosm's preparation protocol, cell counting of pelagic bacteria and measurements of final ibuprofen concentration is available in the Supplementary methods.

3.4 | *Statistical analysis*

Analyses were carried out using R software (R Core Team, 2018). First, a balanced 2–Way ANOVA test was performed to test the effect of glucose availability, density of bacterial predator and its interaction on bacteria population growth. Then, a post–hoc Tukey test was finally applied to compare which specific treatments differ significantly. Secondly, multiple linear regression techniques were used to build an inferential test to analyse the percentage of ibuprofen consumption depending on cell density of pelagic bacteria, glucose availability (potential competitor with ibuprofen uptake), levels of predator density and their interaction. Backward model selection based on hypothesis testing was applied to find the optimal model. To do so, a full model containing all the variables and first–order interaction was fitted initially. Subsequently, non–significant terms were dropped sequentially using the *drop1* function within R, and re–fitting the reduced model at each step. Dependency of structure of the residuals with the microcosm–plate (two 96 multi–well plates were used) was not detected and therefore it was not included as a correcting factor in the

previous ANOVA test and inferential model. ANOVA test and regression model validation (normal distribution, homogeneity of the model residuals and dependency of residuals with variables included and not included in the analysis) was analysed graphically following Zuur et al. (2009) to verify the underlying assumptions. Numerical results from ANOVA test and regression model are available in the Tables S4.2 and S4.3.

4 | Results

We found that population growth of bacterial degraders resulted in high consumption of ibuprofen (the bacteria had been chosen because they can degrade ibuprofen) but this pattern was highly dependent on the level of predator density. The resulting interaction revealed an important trade–off behind the functioning of the hyporheic bioreactor.

First of all, from our ANOVA results, we found that glucose availability significantly promoted density of pelagic bacteria (ANOVA test: $F_{4,100} = 147.1$, $P < 0.001$; see Table S4.2 for ANOVA details) and it was notably higher in treatments with elevated concentration of glucose (Fig 4.1a). ANOVA test also detected the presence of the bacteria predator (*T. pyriformis*) as an important influencing factor on pelagic bacteria population density. An intensification in the predator density level had a significant effect on bacteria population growth (ANOVA test: $F_{4,100} = 135.3$, $P < 0.001$), but not in the linear–fashion observed in the case of glucose. Instead, we observed a convex shaped effect. This hormesis–like effect of predator presence resulted in significantly higher degrader densities at low and medium levels of predation, compared to the control, high and very high predation levels (Fig 4.1b). Not only did glucose and predator density levels determine pelagic bacterial population

growth, but also the two predictors showed a strong synergic interaction (i.e. the combined effect was more than the sum of its parts; Fig 4.1c) on this response ($F_{16,100}$ = 3.8, P < 0.001). As a consequence of this interaction, cell density of pelagic bacteria was compensated even in treatments of glucose deficit under low and medium levels of predation.

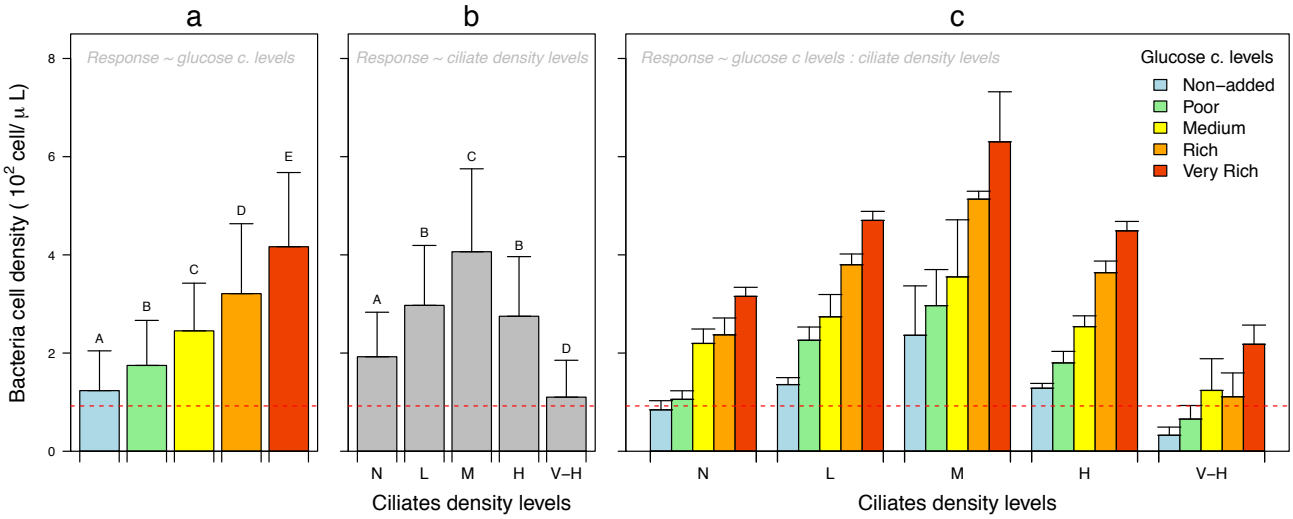

Fig 4.1. Results from the ANOVA test analysing final cell density of pelagic bacteria (mean ± SD) at different glucose concentration levels (**a**), different ciliate density levels (**b**) and in the total different treatments combinations (**c**: interaction between factors) after 24 h experiment. Note that as consequence of the synergic interaction between factors, response increases notably in panel–c. The red dotted line marks the initial bacterial cell density (0.92 × 10^2 cell/µL). Treatments where responses are not significantly different after post–hoc Tukey test (P–value > 0.05) are indicated with the same letter in each panel (in panel a and b). Glucose concentration levels (glucose c. levels) correspond to 0.00 (none–added, control), 0.08 (poor), 0.31 (medium), 1.25 (rich) and 5.00 (very rich) mg/mL. Ciliate density levels correspond to 0.00 (N = none), ~0.41 (L = low), ~1.62 (M = medium), ~ 6.50 (H = high), ~ 26.00 (V–H = very high) × 10^4 ind/mL.

In the second place, the increase in cell density of pelagic bacteria resulted in an efficient consumption of ibuprofen and the regression fitted relatively well the observed values (Fig 4.2a). Glucose availability, even given its strong and positive effect on bacterial population development, did not show a significant effect on ibuprofen

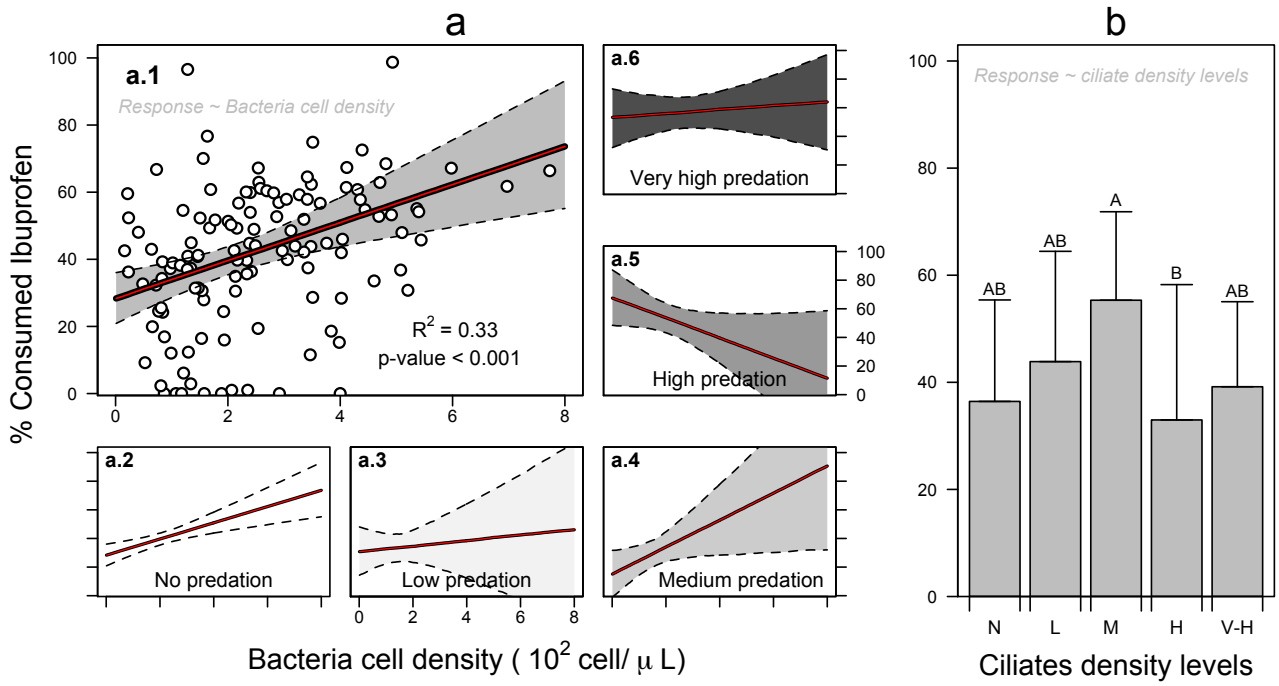

Fig 4.2. Results from the fitted regression model for the percentage of consumed ibuprofen. White dots represent observed percentage values of consumed ibuprofen (response). Grey shaded areas on the regression lines (in red) represent the 95%CI. (**a.1**) Predicted effect of the covariate (bacteria cell density) by itself on the response. (**a.2–a6**) Variation in the relationship between the covariate and the response as consequence of the interaction with predator density factor (5 ciliate density levels). (**b**) Bar–plot showing the mean percentage of consumed ibuprofen (± SD) at different predator density levels. Factor labels correspond with those attached in Fig 4.1. Levels where responses are not significantly different after post–hoc Tukey test (P–value > 0.05) are indicated with the same letter. Shaded areas on the regression lines (in red) represent the 95%CI of the continuous covariate.

consumption (P > 0.05) and this factor was removed from the regression equation during the model selection routine. Although ibuprofen consumption showed a clear positive tendency with rising levels of glucose availability, residuals from the regression model did not show any dependency structure with this factor following its removal (Fig S4.2) i.e. glucose availability provided the same information as cell density of bacteria degrader in the model. In contrast, predator density was preserved as an important factor in the regression equation after model selection routines. This factor also showed a significant hormesis–like effect on EOC consumption (Regression model: $F_{4,115}$ = 3.1, P = 0.02; Table S4.3), which clearly corresponded with its effect on

bacterial population growth (Fig 4.2 b). However, predator density levels showed a significant interaction with the regression covariate (Regression model: $F_{4,115}$ = 22.6, P < 0.001). In fact, removing the predator density factor from the regression equation produced a strong reduction in robustness and explanatory capability (R^2 model without predator density factor = 0.13). As a consequence of this interaction, the relationship between cell density of the bacterial degrader and ibuprofen consumption was much stronger (more pronounced slope in the fitted line) at medium levels of predator density than in the rest of the treatments (Fig 4.2a.2–a.6). In this manner, predator density did not just determine the bacteria population growth, but also the efficiency in the EOC consumption.

5 | Discussion

We were able to show, in a controlled experimental setting, that a model strain of pelagic bacteria isolated from the HZ efficiently removed a model EOC (ibuprofen) from the medium. EOCs removal in the HZ is driven by complex processes and extrapolating our results to natural systems, we expect that the highest bioreactor efficiency will occur when density of pelagic bacteria cells is high and when bacteria predators are present at densities that are sufficient to stimulate bacterial activity but not at such high densities as to over–predate them (Fig. 3). Moderate levels of predator density therefore boost both pelagic bacteria population growth, and their efficiency as EOC degraders. This outcome may be explained by the selective consumption of less active bacteria (Shapiro *et al.* 2010), by the predator effect on bacteria dispersal in the medium (as a consequence of swimming around), resulting in better exposure to glucose and the EOC (Otto *et al.* 2017) and the predation–induced recycling of nutrients (microbial loop) (Shapiro *et al.* 2010). The ciliate species used in the experiments is also

able to utilise glucose directly, i.e. it competes with bacteria for glucose when it is abundant. This potential competition might also encourage bacteria degraders switch to ibuprofen as an energy source when glucose becomes scarce. Importantly, the 'right' level of predator density can compensate for low nutrient availability in terms of EOC degradation (Fig 4.2 and 4.3).

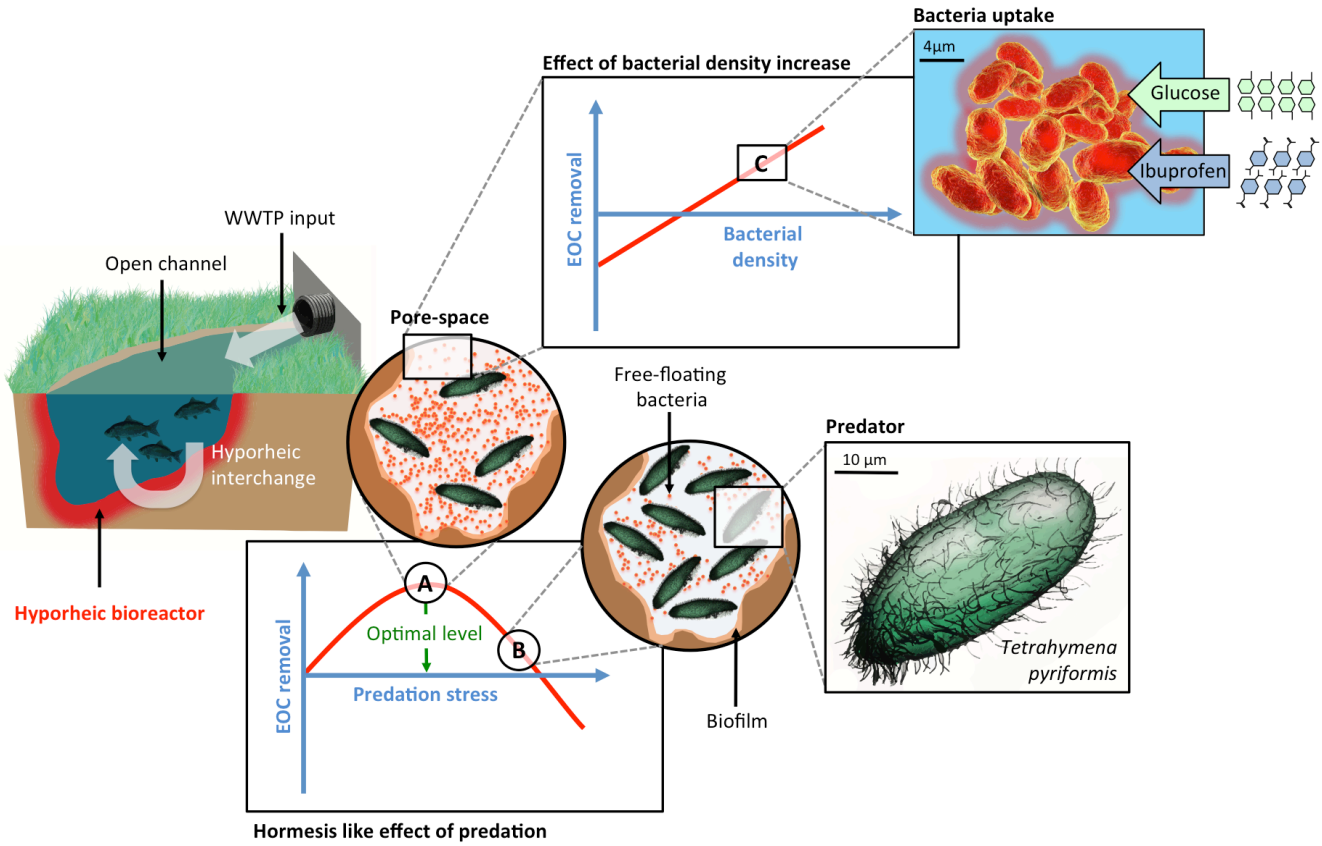

Fig 4.3. Conceptual depiction of the EOCs removal efficiency by the hyporheic bioreactor under different scenarios of predation stress and along the gradient of cell density of pelagic degrader. Wastewater treatments plant input is the main transport pathway of emerging organic contaminants (EOCs) in streams and rivers. As consequence of the hyporheic interchange, dissolved EOCs penetrate into the pore–space of streambed sediments, where active bacterial consortiums, including pelagic bacteria and biofilm, could consume them. The surface water inflow in the pore–space is also related with deliveries of DOC, which boosts the bacterial population growth (C). Despite the input of DOC is not directly related with EOCs consumption, its positive effect on bacteria population grow could promote it. Besides, EOCs consumption efficiency is subjected to the hormesis–like effect of predation on the pelagic bacteria. (A) There is an optimal range of predation that stimulates density of pelagic bacteria and EOCs degradation until (B) the system is overloaded and the consumption of bacteria is decompensated. As result, the EOCs consumption can be maintained in the hyporheic bioreactor even under a scenario of nutrient deficit

Our findings demonstrate that cell density of pelagic bacteria is a variable that could be a conservative predictor of EOC removal for field scenarios (Fig 4.3). In contrast, the weaker relationship between glucose and EOC consumption observed here could result from the existence of a trade–off between its positive effect on bacterial population growth and its role as a potential substrate competitor with the EOC. Thus, our findings predict that the 'right' level of predator density could have a greater impact on the efficiency of EOC consumption by bacteria than nutrient availability, and it could even compensate for low nutrient availability during EOC degradation. Here, we artificially increased the carrying capacity of the predator and, as a consequence, the predator stress on pelagic bacteria. However, under healthy natural conditions, regulating mechanisms (i.e. second level predation, intra– and interspecific competition) tend to keep the exponential growth capacity of predator populations in check (Barabás *et al*. 2017). Therefore, it might be expected that the optimal level of predator stress reported here would be maintained through biotic and abiotic controlling factors in natural systems.

Our study is the first to assess the role of predators on biodegradation of EOCs in natural bioreactors and more studies in different systems are needed in order to generalise our results (i.e. soil systems). In addition, future research should also consider the multilevel trophic interactions that take place within the natural communities to achieve a more realistic perspective of natural bioreactors functioning. Furthermore, we emphasize that our approach is focussed on pelagic bacteria. It is very probable that, under natural conditions, the strain of bacteria we used also forms biofilms attached to the surface of the sediment resulting in potentially different ecological processes.

Here we demonstrate the functional importance of nutrient loading and predator presence as important gears in the performance of biologically active interfaces such as the hyporheic bioreactor in the streambed system. In light of our results, promoting water interchange between the open channel and the HZ, and consequently the nutrient (as DOC) and EOC loading in the streambed pore space, is a suitable strategy to mitigate the entry of EOCs in freshwater systems. Our findings also highlight the importance of preserving natural predator–prey dynamics in order to promote ecosystem services upon which human well–being depends (Soliveres *et al.* 2016).

6 | Acknowledgments

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7 | Appendix chapter 4

Supplementary Methods

Bacteria strain isolation and preparation – Water and sediment samples for isolation of bacteria were collected in September 2015 from the HZ of River Erpe (Berlin, Germany), 700 m downstream from the effluent input of the municipal wastewater treatment plant Münchehofe (Rutere & Horn, unpublished). An ibuprofen biodegradation potential of the hyporheic sediment was initially confirmed with ibuprofen supplemented (400 µM), and heat-sterilized sediment slurry as well as sterilized river water as abiotic controls where no degradation was observed within 35 days at 15 °C. To enrich for ibuprofen degraders, approximately five grams wet

sediment was added to 35 ml oxic mineral salt medium without glucose (sugarless-growth medium; Table S4.3) and supplemented with 400 μM 98% analytical grade ibuprofen (Sigma-Aldrich, Steinheim, Germany). Enrichments were incubated in the dark at 15°C under oxic conditions, and ibuprofen was re-fed upon depletion. Subsequently, a serial dilution was performed in the same medium with ibuprofen, incubated, and the enrichment culture from 10-6 streaked onto the same medium solidified with 1% agar. Isolated colonies were then transferred to liquid medium and tested for ibuprofen degrading capabilities. An ibuprofen degradation positive colony was purified via repeated transfer on solid media containing ibuprofen as the sole significant carbon and energy source. Combined evidence including 16S rRNA gene sequence analysis suggested a novel bacterial strain of Alphaproteobacteria using ibuprofen as a sole carbon and energy source.

Microcosm's preparation protocol – Firstly, microcosms were filled with 1mL of sugarless-growth medium containing different concentrations of glucose. Different levels of glucose availability were obtained following a serial dilution: 0.00 (control), 0.08 (poor), 0.31 (medium), 1.25 (rich) and 5.00 (very rich) mg/mL glucose in 2 mL final volume. Secondly, 0.25 mL of concentred dilution of ibuprofen in ddH2O was pipetted onto all the microcosms obtaining 0.08 mg/mL ibuprofen in 2 mL final volume. Due to the low solubility of ibuprofen in water, a stock solution was prepared by dissolving ibuprofen into methanol, and evaporating the methanol under a fume hood at room temperature. Then, sterilised distilled water was added. Thirdly, 0.5 mL inoculums with the different levels of ciliate density were added into the microcosms. Immediately before ciliate inoculation, from the fresh stock with a carrying capacity of population density, ciliates were harvested by centrifugation at 300 rpm for 7 min at 0 °C and re-suspended three times in sugarless-growth medium. The last re-suspension

was performed using a four times lower final volume of growth medium (giving a final ciliate density four times higher than the carrying capacity) Different levels of ciliate density were then obtained by following a serial dilution: 0.00 (control), ~0.41 (low), ~1.62 (medium), ~ 6.50 (high), ~ 26.00 × 10^4 (very high) ind/mL in 2 mL final volume. Finally, 0.25 mL of bacterial inoculum was quickly added to all the microcosms with a multichannel pipette. Bacterial inoculum was transferred from a mature bacterial stock, which had been previously centrifuged at 15000 rpm for 1 min and re-suspended three times in sugarless-growth medium. An initial bacterial count from the fresh inoculum was measured using flow cytometer techniques (see details below). Immediately after bacterial inoculation, pH was checked and microcosms were covered with a semipermeable membrane. Then, microcosms were incubated at 15 °C culture chambers for 24 h.

Cell counting of pelagic bacteria – After 24 h, 200 µL of PicoGreen dye solution (Quant-iT™ PicoGreen™ dsDNA Assay Kit, Sigma-Aldrich) were added to the microcosms in order to stain the DNA of living cells. Microcosms were then incubated at 4°C for 15 min. Then 1 mL subsamples were extracted for bacterial counting while microcosms with the rest of solution were immediately frozen for subsequent measurements of ibuprofen concentration. Subsamples for bacterial counting were centrifuged three times at 15000 rpm for 1 min, re-suspended in PBS buffer and fixed using 4% paraformaldehyde. Fixed subsamples were filtered using cellulose acetate membrane filters (0.45 µm) to remove ciliates from the medium. Finally, bacterial counts were measured using an Accuri C6 flow cytometer (BD Biosciences) on slow with a forward scatter-H of 8000 and a side scatter-H of 2000.

Measurements of final ibuprofen concentration – Ibuprofen was quantified using liquid chromatography-targeted tandem mass spectrometry (LC-MS/MS/MS). 1 mL

subsamples form the microcosms and external ibuprofen standards were spiked and subsequently extracted using solid phase extraction (SPE) cartridges (Strata-X columns 500 mg 6mL−1; Phenomenex). Of the SPE eluates, 10 µl were injected. Separation was achieved on a Water Acquity H Class UPLC system using a Waters HSS T3 UPLC column (2.1 mm X 100 mm, 1.8 µm particle size, equipped with a HSS T3 VanGuard pre-column maintained at 45°C. The UPLC system used 0.1% formic acid (in water) as phase A and 0.1% formic acid (in acetonitrile) as phase B. The gradient was 1% B for 1 min, then up to 70% B at 2 min, 95% B at 3.3 min, held to 4.1 min, when the system switched back to 1% B, which was held for 0.9 min. Mass spectra were obtained on a Waters TQSmicro triple quad mass spectrometer with electrospray ionisation in negative mode. The source potential was 2.4kV and the source held at 450°C with a desolvation gas flow of 650L/hr. Monitored transitions (parent to daughter) were 205.3 to 161.3 (cone energy 30V, collision energy 12V) and 205.3 (cone energy 30V, collision 1V), with the former being used for quantification. For data analysis and baseline correction, spectra were imported into Matlab software and compound levels were quantified. External ibuprofen standards were used to generate standard curves, which were used to calculate the concentrations.

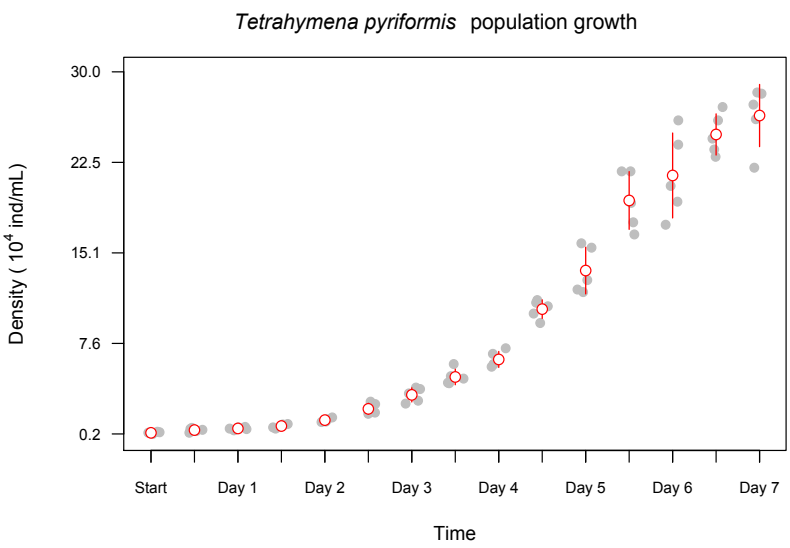

Fig S4.1. *Tetrahymena pyriformis* population growth measured every 12 h. cultured in 2% protease peptone medium at 15 °C and pH 7. Five subsamples from the stock were measured every 12 h. (grey dots). White dots are the mean value and intervals are the standard deviation of the ciliate population density every 12 h.

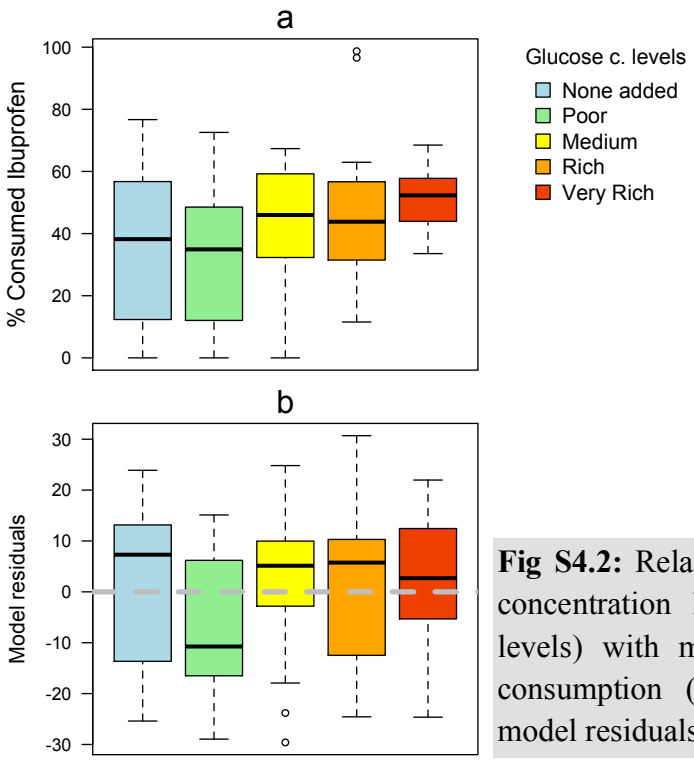

Fig S4.2: Relationship of glucose concentration levels (Glucose c. levels) with measured ibuprofen consumption (a) and regression model residuals (b).

Table S4.1: Sugarless-growth medium.

Mineral salt	**Final concentration (mg/L)**
KH_2PO_4	10.0 × 10
NaCl	8.0
NH_4Cl	8.0
$MgCl.6H_2O$	1.0
$CaCl_2.2H_2O$	0.2
Trace elements	
$C_6H_6NO_6Na_3$	750.0
$MnSO_4.H_2O$	250.0
Fe $SO_4.7H_2O$	50.0
CO $(NO3)_2$. $6H_2O$	50.0
$ZnCl_2$	50.0
H_2SeO_4-96% solution	50.0
$NiCl_2.6H_2O$	25.0
$CuSO_4.5H_2O$	5.0
AlK $(SO_4)4$. $12H_2O$	5.0
H_3BO_3	5.0
$Na_2MoO_4.2H_2O$	5.0
$Na_2WO_4.2H_2O$	5.0
Vitamin Solution	
Lipoic acid (D,L-6,8 Thiocitic acid)	12.5
Riboflavin	12.5
Thiamine HCl	12.5
Ca-D-Pantothenate	12.5
Cyanocabolamin	12.5
p-Aminobenzoic acid	12.5

Continue table S4.1

Nicotinic acid	12.5
Biotin	5.0
Folic acid	5.0
Pyridoxal.HCl	5.0

Table S4.2: ANOVA table testing cell density of pelagic bacteria as response of glucose availability (*Glucose*; 5 levels factor), ciliate density (*Predation*; 5 levels factor) and its interaction.

	Df	Sum sq	Mean sq	F-value	p-value
Glucose (Factor 1)	4	1357202.0	339300.0	147.1	< 0.01
Predation (Factor 2)	4	1248923.0	312231.0	135.3	< 0.01
Factor 1× Factor 2	16	141003.0	8813.0	3.8	< 0.01
Residuals	100	230686.0	2307.0		

Table S4.3: ANOVA table testing percentage of ibuprofen consumption as response of cell density of pelagic bacteria (continuous covariate), ciliate density (*Predation*; 5 levels factor) and its interaction.

	Df	Sum sq	Mean sq	F-value	p-value
Bacteria density (Covariate)	1	7183.0	7183.2	22.6	< 0.01
Predation (Factor 1)	4	3974.0	993.6	3.1	0.02
Covariate × Factor 1	16	6485.0	1621.1	5.1	< 0.01
Residuals	115	36601.0	318.3		

8 | References

Altermatt, F. *et al.* Big answers from small worlds: a user's guide for protist microcosms as a model system in ecology and evolution. *Methods Ecol. Evol.* **6**, 218–231 (2015).

Barabás, G. *et al*. Self-regulation and the stability of large ecological networks. *Nat. Ecol. Evol.* **1**, 1870 (2017).

Bardini, L. *et al.* Nutrient cycling in bedform induced hyporheic zones. *Geochim. Cosmochim. Acta,* **84**, 47–61 (2012).

Battin, T. J. *et al.* Contributions of microbial biofilms to ecosystem processes in stream mesocosms. *Nature*, **426**, 439 (2003).

Battin, T. J. *et al*. The ecology and biogeochemistry of stream biofilms. *Nat Rev Microbiol*. **14**, 251 (2016).

Bengtsson, G. Growth and metabolic flexibility in groundwater bacteria. *Microb Ecol*. **18**, 235–248 (1989).

Cassidy–Hanley, D. M.in *Methods in Cell Biology: Tetrahymena thermofila* (ed. Collins, K.) 239–276 (Academic Press, Amsterdam, 2012).

Camesano, T. A. & Logan, B. E. Influence of fluid velocity and cell concentration on the transport of motile and nonmotile bacteria in porous media. *Environ. Sci. Technol*. **32**, 1699–1708 (1998).

Fuller, M. E. *et al.* Examining bacterial transport in intact cores from Oyster, Virginia: effect of sedimentary facies type on bacterial breakthrough and retention. *Water Resour. Res*. **36**, 2417–2431(2000).

Evgenidou, E. N. *et al*. Occurrence and removal of transformation products of PPCPs and illicit drugs in wastewaters: a review. *Sci. Total Environ*. **505**, 905–926 (2015).

Febria, C. M. *et al.* Bacterial community dynamics in the hyporheic zone of an intermittent stream. *ISME J.* **6**, 1078 (2012).

Findlay, S. Importance of surface‐subsurface exchange in stream ecosystems: The hyporheic zone. *Limnol Oceanogr*. **40**, 159–164 (1995).

Findlay, S. (2010). Stream microbial ecology. *J. N. Am. Benthol. Soc*. **29**, 170–181 (2010).

Flemming, H. C., & Wingender, J. The biofilm matrix. *Nat. Rev. Microbiol.* **8**, 623 (2010).

Foulquier, A. *et al.* Response of sediment biofilm to increased dissolved organic carbon supply in groundwater artificially recharged with stormwater. J. Soil. Sediment. **11**, 382–393 (2011).

Gantzer, C. J. *et al.* Mass transport to streambed biofilms. Water Res. **22**, 709–722 (1988).

Han, S. *et al.* Endocrine disruption and consequences of chronic exposure to ibuprofen in Japanese medaka (Oryzias latipes) and freshwater cladocerans Daphnia magna and Moina macrocopa. *Aquat. Toxicol.* **98**, 256-264 (2010).

Harvey, R. W. & Garabedian, S. P. Use of colloid filtration theory in modeling movement of bacteria through a contaminated sandy aquifer. *Environ. Sci. Technol*. **25**, 178–185 (1991).

Heckmann, L. H. *et al.* Chronic toxicity of ibuprofen to Daphnia magna: effects on life history traits and population dynamics. *Toxicol. Lett.* **172**, 137–145 (2007).

Lewandowski, J. *et al.* Fate of organic micropollutants in the hyporheic zone of a eutrophic lowland stream: results of a preliminary field study. *Sci. Total Environ*. **409**, 1824–1835 (2011).

Malaj, E. *et al.* Organic chemicals jeopardize the health of freshwater ecosystems on the continental scale. *Proc. Natl. Acad. Sci. U.S.A.* **111**, 9549–9554 (2014).

Mulholland, P. J. *et al.* Stream denitrification across biomes and its response to anthropogenic nitrate loading. *Nature*, **452**, 202 (2008).

Otto, S. *et al.* Effects of predation and dispersal on bacterial abundance and contaminant biodegradation. *FEMS Microbiol. Ecol.* **93**, fiw241. (2017).

Pal, A. *et al.* Impacts of emerging organic contaminants on freshwater resources: review of recent occurrences, sources, fate and effects. *Sci. Total Environ*. **408**, 6062–6069 (2010).

Peralta–Maraver, I. et al. Interplay of hydrology, community ecology and pollutant attenuation in the hyporheic zone. *Sci. Total Environ*. 610, 267–275 (2018).

R Core Team (2018). *R: A language and environment for statistical computing.* R Foundation for Statistical Computing, Vienna, Austria. http://www.R–project.org/.

Radke, M. *et al.* Fate of the antibiotic sulfamethoxazole and its two major human metabolites in a water sediment test. *Environ. Sci. Technol*. **43**, 3135–3141 (2009).

Schulz, M. *et al.* Transformation of the X–ray contrast medium iopromide in soil and biological wastewater treatment. *Environ. Sci. Technol*. **42**, 7207–7217 (2008).

Schwarzenbach, R. P. *et al.* The challenge of micropollutants in aquatic systems. *Science*, **313**, 1072–1077 (2006).

Shannon, M. A. *et al.* Science and technology for water purification in the coming decades. *Nature*, **452**, 301 (2008).

Shapiro, O. H. *et al.* Bacteriophage predation regulates microbial abundance and diversity in a full-scale bioreactor treating industrial wastewater. *ISME J.* **4**, 327 (2010).

Soliveres, S. *et al.* Biodiversity at multiple trophic levels is needed for ecosystem multifunctionality. *Nature*, **536**, 456. (2016).

Stamm, C. *et al.* Unravelling the impacts of micropollutants in aquatic ecosystems: interdisciplinary studies at the interface of large–scale ecology. *Adv. Ecol. Res*. **55**, 183–223 (2016).

Stegen, J. C. *et al.* Groundwater–surface water mixing shifts ecological assembly processes and stimulates organic carbon turnover. *Nat. Commun*. **7**, 11237 (2016).

Verlicchi, P. *et al.* Occurrence of pharmaceutical compounds in urban wastewater: removal, mass load and environmental risk after a secondary treatment—a review. *Sci. Total Environ*. **429**, 123–155 (2012).

Vörösmarty, C. J. *et al.* Global threats to human water security and river biodiversity. *Nature*, **467**, 555 (2010).

Wen Y. *et al.* Organic pollution of rivers: Combined threats of urbanization, livestock farming and global climate change. *Sci. Rep*., **7**, 43289 (2017).

Zuur, A. F. *et al. Mixed Effects Models and Extensions in Ecology with R*. (Springer, New York, 2009).

Chapter 5 | Predicting leaf litter decay in the streambed: response to system compartmentalization and involvement of the whole assemblage of organisms

Ignacio Peralta–Maraver[1*], Dan Perkins[1], Murray Thompson, Katarina Fussman[1], Julia Reiss[1], Anne Robertson[1].
[1]Department of Life Sciences, Roehampton University, London, UK
[2]Centre for Environment, Fisheries and Aquaculture Science, Lowestoft Laboratory, Lowestoft, UK
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1 | Abstract

1. Leaf litter processing in the streambed is an important pathway in organic carbon cycling and energy transfer in the biosphere. However the rate at which this process occurs might differ between the benthic (BZ) and the hyporheic (HZ) zone. Furthermore, several processes mediated by a wide range of organisms might precede the leaf litter breakdown. Nevertheless, most research on this process is limited to the BZ and involves only a small fraction of taxa inhabiting the streambed.
2. Here we inferred different leaf litter breakdown rates by combining two assays; the cotton–strips assay and the tea bag index (TBI). Then, we modelled these assays as a response of the streambed compartment and the biological features of the streambed assemblage. We followed a regional–scale approach along 30 different low land rivers and included a large range of organisms (Prokaryota, Protozoa and Eumetazoa invertebrates).
3. The rate of leaf litter processing was always higher in the BZ than in the HZ. Furthermore, the biomass of all the studied groups, α–diversity of Eumetazoa invertebrates and functional diversity of Prokaryota were important predictors that were positively related with the decay rates.
4. Our findings confirm that, under permanent flow conditions, the BZ is more important in leaf litter processing, while the HZ acts as a sink of carbon. Our findings also demonstrate that the bioreactor ability of the streambed is sustained and maintained by diverse and active assemblages and that all size categories play an important role.

2 | Introduction

Globally, terrestrial plants produce approximately 120 billion tons of organic carbon annually (Beer *et al.* 2010) and more than 90% of this production escapes from herbivores (Gessner *et al.* 2010). Thus, the breakdown of plant litter is an essential biosphere–scale ecosystem process (Boyero *et al.* 2011, Datry *et al.* 2018). Streams and rivers, despite covering less than 1% of the Earth´s surface, contribute significantly to litter breakdown, and by extension to the global carbon cycle (Battin *et al.* 2008, 2009, Borrows *et al.* 2017). Stream sediments are sites of intense leaf litter processing (streambed as a natural bioreactor of allochtonous organic carbon) because this material provides a major source of energy for the diverse and active biota that occurs in stream beds (Vannote *et al.* 1980, Gessner *et al.* 1999, Sabater *et al.* 2008). Therefore, research on the mechanism behind litter processing in running water is of great interest in geological and biological sciences. Traditionally, this ecological process has been assessed by applying leaf litter assays in streambed studies (Webster & Benefield 1986). However, this approach presents considerable limitations as a standardized method in large–scale studies (Tiegs *et al.* 2007). For this reason, researchers have begun to use artificial cellulose objects, such as cotton–strips, to obtain standardised global scale decomposition data in the streambed based on the loss of tensile strength (Tiegs *et al.* 2007, Woodward *et al.* 2012, Tiegs *et al.* 2013). Another recent approach is to gather data on leaf litter processing using commercially available tea bags as highly standardised test kits. This method, also known as the tea bag index (TBI), is based on the use of two tea types with contrasting decomposability (green tea and rooibos tea). The acquired TBI consists of two parameters describing global litter decay coefficient (*K*) and leaf litter stabilisation factor (*S*: the proportion of leaf litter that escapes from processing and becomes recalcitrant as a consequence of environmental factors)

(Keuskamp *et al.* 2013). This method also allows the separate measurement of the decay coefficients of each tea type based on loss in weight after an incubation period.

The rate of leaf litter processing in the streambed depends on several agents, including the dissolution of labile compounds (leaching), microbial conditioning, consumption, fragmentation and environmental abrasion (Webster & Benfield 1986). During this process, many taxonomic groups of the assemblage are involved. Prokaryotic (Archea and Bacteria) and fungal consortia drive the initial litter decomposition processes (Gulis & Suberkropp 2003). Then, Protozoa (including ciliates, flagellates and amoeba) inhabiting the sediment pore space might stimulate prokaryotic population growth and activity (Risse–Buhl *et al.* 2012). Previous microcosm research has found that the decay rate of leaf litter can be three to four times higher when bacterivorous Protozoa were present compared with treatments in which they were excluded (Ribblett *et al.* 2005). As a result of the initial microbial processing, so–called leaf–conditioning (Gessner & Chauvet 1994) increases the palatability and quality of leaf litter as a food resource for invertebrate shredders (Gonçalves *et al.* 2007, Foucreau *et al.* 2016). In addition to direct consumption, Eumetazoan invertebrates might also intervene indirectly in the breakdown process. Life activities of these organisms (digging and removing the sediment) produce bioturbation and bioirrigation phenomena in the streambed (Baranov *et al.* 2016a, Baranov *et al.* 2016b), which could enhance prokaryotic activity and oxidation of organic matter (Kristensen *et al.* 2012, Baranov *et al.* 2016b). Thus, it can be expected that a large range of taxa inhabiting the streambed, not just biofilms and shredders, might be involved in some way during leaf litter processing. However, the role of the whole assemblage of interstitial organisms in streambed processes and services is little known (Boulton 2000, Cornut *et al.* 2010, Navel *et al.* 2010 Marmonier et al. 2012, Peralta–Maraver et al. 2018a). Studies that

include all the components of the streambed assemblage are very rare (Reiss & Schmid–Araya 2008, Reiss & Schmid–Araya 2010, Peralta–Maraver *et al.* 2018a, Peralta–Maraver *et al.* 2018b).

Furthermore, the streambed is a compartmentalized system that is often divided into two zones that differ in depth. The benthic zone (BZ) is located in the upper centimetres of the streambed, in direct contact with the stream water flow and exposed to light whereas the hyporheic zone (HZ) encompasses the volume of sediment beneath the BZ where surface water interacts with groundwater (Findlay 1995, Boulton *et al.* 1998, Robertson & Wood 2010, Battin *et al.* 2016). These two compartments support discrete communities that differ in their structure and composition (Peralta–Maraver *et al.* 2018b). The processing of leaf litter also seems to differ between the two compartments. Majority of leaves falling into streams and rivers are trapped by streambed structures, mostly cobbles and woody debris, forming leaf packs that are processed in the BZ (Peralta–Maraver *et al.* 2011). However, a substantial part of the total leaf litter entering streams and rivers is buried and stored in the HZ as a consequence of storm events, flooding and sediment movements (see Cornut *et al.* 2010). Once in the HZ the rate of leaf litter decomposition is markedly reduced in comparison with the BZ (Cornut *et al.* 2010, Danger *et al.* 2012). Accordingly, it now seems likely that the benthic (*benthos*) and hyporheic communities (*hyporheos*) differ in their ability to process leaf litter although this has not yet been evaluated and the links between streambed compartmentalization (BZ *vs* HZ), assemblages of interstitial organisms (including benthos and hyporheos), microbial activity, and leaf litter processing in the streambed remain poorly studied. Accordingly, large–scale studies incorporating the variables described above will notably improve the current understanding of this important ecosystem process. By extension, it will also allow the

construction of suitable predictive models that will enable us to infer leaf litter processing under different scenarios of distribution, biomass, diversity and activity of the assemblage.

Here we study the main factors driving the leaf litter breakdown rate in the streambed following a regional–scale approach and involving a large range of taxonomic groups (Prokaryota, Protozoa and Eumetazoa invertebrates). Firstly, we carried out a survey to determine how biomass and α–diversity of Protozoa and Eumetazoa invertebrates, as well as biomass, functional diversity and potential metabolic activity of Prokaryota (specifically aerobic metabolic potential to use carbon sources) differ between streambed compartments across 30 different rivers. From this descriptive stage in our research, we characterised the little–known biological features of streambed bioreactor. Subsequently, we investigated the role of these variables and the streambed compartment on the leaf litter processing by combining the cotton–strips assay and TBI index, and building inferential models for the decay coefficients of the different substrata and *S*. Based on the obtained models, we aimed to test the following hypotheses:

1. The top layers are the most active part of the streambed bioreactor and therefore organic carbon processing is expected to be higher in the BZ than in the HZ. Consequently, the streambed compartment will be an important predictive factor in our inferential models, emphasising that the BZ and the HZ are indeed different environments, which differ not only in their biological features, but also in the ecological processes that take place in them.
2. The whole assemblage of organisms is involved in leaf litter processing in the streambed. Thus biomass and α–diversity of Protozoa and Eumetazoa invertebrates, as well as biomass, functional diversity and potential metabolic

activity of Prokaryota will be detected as significant covariates in our inferential models with a positive effect on the responses demonstrating the necessity of including all taxonomic groups when assessing ecosystem processes in the streambed.

3. The effect of the studied biological variables (models covariates) on the leaf litter processing is stronger in the BZ than in the HZ. As result, our models will include significant interaction terms between some of the biological variables and the streambed compartment suggesting that benthos and hyporheos are discrete communities that differ in their contribution to this ecosystem process.

3 | Methods

3.1 | *Survey design and samples processing*

We studied rates of leaf litter breakdown in the BZ and the HZ of 30 lowland streams from 10 different catchments located across England and Wales in the UK (Fig. 5.1). The streams covered a wide range of latitude, productivity and pH, which allowed us to integrate important environmental gradients into our analysis (detailed characterisation of the study sites is available as Table S5.1) and generalize our results. The study units consisted of coarse mesh–packages (mesh size = 0.5 cm) containing three different substrata: two commercially available tetrahedron–shaped synthetic tea–bags (Lipton; mesh size = 0.25 mm) and a single cotton–strip (8.0 cm × 2.0 cm; made of 100% unbleached cotton, 96% cellulose). One of the tea–bags contained dried leaves of green tea (89%) and the other contained dried leaves of rooibos (red) tea (93%). Mesh–packages were designed to retain the experimental organic substratum during the incubation period in the field, but they were also used as standardised colonization containers for streambed organisms. Mesh–packages protecting the three substrata were

fixed in pairs with a rope to an iron rod in the streambed sediments one package was

	System	Catchment	Latitude	Longitude
a	River Coanwath	Eden	54.6000	-2.5044
	River Lyvenet	Eden	54.6147	-2.6200
	River Leith	Eden	54.6141	-2.6195
	Morland Beck	Eden	54.6083	-2.6110
	Howe Beck	Eden	54.6083	-2.5867
b	LI-8	Tywi	52.1640	-3.7498
	LI-3	Tywi	52.1427	-3.7348
	LI-6	Tywi	52.1329	-3.7233
	LI-7	Tywi	52.1294	-3.7498
	GI-1	Tywi	52.1035	-3.8437
c	Stiffkey	Stiffkey	52.9190	0.8872
	Glaven	Glaven	52.9031	1.0631
	Bure	Bure	52.8242	1.2013
	Tat	Wensum	52.8216	0.7466
	Wensum	Wensum	52.7765	0.9501
	Waveney	Waveney	52.4215	1.3566
d	Beberly Brooks	Thames	51.4422	0.2549
	Kennet	Thames	51.4234	1.7166
	Loddon	Thames	51.2922	1.0179
	Lyde	Thames	51.2875	1.0023
	Nadder	Wey	51.2286	0.7982
	Wey	Wey	51.1871	0.6827
	Anton	Test	51.1530	1.4600
	Lamports	Wey	51.1514	0.9664
	Test	Test	51.1394	1.4735
	Oakhanger	Wey	51.1164	0.8990
	Deadwater	Wey	51.1074	0.8503
	Broadston Stream	Medway	51.0887	0.0574
	Loan Oak	Medway	51.0765	0.1033
	Old Lodge	Medway	51.0454	0.0788

Fig 5.1. Locations of the study systems in the United Kingdom including the catchment area to which they belong, latittude and longitude.

buried at 2 cm (BZ) and the other at 20 cm (HZ) depth. Mesh package deployment began on 25th October 2016 during peak leaf fall. Three pairs of packages were deployed along a 50m section per study site (3 replicates per zone, 180 packages in total) using a piezometer pipe with a conical tip fitted on the bottom. At each study site, a temperature datalogger (IButton DS1922L, accuracy of ±0.5°C) was attached to one of the mesh–packages at 20 cm depth, which recorded temperature every 10 minutes in the HZ. Spot surface–water temperature measurements were recorded during collection of samples and the exact time was recorded, so that it was possible to compare them with the equivalent hyporheic values. Afterwards, a simple linear regression of surface temperature as response of the equivalent time measurements in the HZ was applied to infer variation of temperature in the BZ during the study period (Fig S5.1).

After 29–61 days of incubation (incubation time differed between sites; Table

S5.1), packages were removed carefully from the field using a hand shovel, kept in 50–ml falcon vials filled with autoclaved mineral water and returned to the laboratory using an ice–chilled cooler. Subsequent measurements of biomass required the mesh–package volume, this was determined as the difference between the total vial volume and the added water volume. Samples were processed in the laboratory within 48 h. of collection. Falcon vials containing mesh–packages were shaken continuously for 1 min at 2500 rpm using a compact vortex shaker (SciQuip Vortex Mixers). Immediately after shaking, 10 ml water was collected with a pipette. From the collected water, 5 ml were filtered using cellulose acetate membrane filters (0.045 µm) to remove Protozoa and Eumetazoa invertebrates from the medium for later measurements of prokaryote biomass diversity and potential activity. The remaining water was kept unfiltered to process Protozoa. Both filtered and unfiltered water samples were stored in sterilised conditions at 4 °C and processed during the 48 h interval after sampling. The remaining content of the vials was rinsed through a 40–µm sieve. The mesh–packages were opened over the sieve in order to wash the tea–bags and cotton–strips. Tea–bags and cotton–strips were stored and the remaining sieve contents were preserved in formalin 4% containing Bengal–rose stain so that invertebrates could be processed at a later time. Tea–bags were dried at 60 °C during 48 h and dry weight of content measured using an electric scale (accuracy of 0.1 µg). Cotton–strips were soaked in 70% ethanol to inhibit microbial activity during storage (Tiegs *et al.* 2007), then air dried, and stored individually in paper envelopes. Tensile strength of all cotton–strips was measured with an Instron Series IX tensiometer (Instron Corporation,Canton, Ohio) at 20 °C and 65% relative humidity in a climate–controlled room. Mean and standard deviation of pre–incubation tensile strength (631.0 ± 17 kg) was measured using 5 new cotton–strips.

3.2 | *Biomass and diversity of Protozoa and invertebrates*

Protozoa, including ciliates, amoeba and flagellates, from the stored unfiltered water were identified and counted alive under an Olympus BX50 microscope. Ciliates and amoeba sub–samples were processed using a Sedgewick rafter counting cell chamber (1 ml volume; Pyser–SGI limited, Edenbridge, United Kingdom), while flagellates were processed using a Neubauer cell counting chamber. Ciliates were identified to sub–class using identification keys (Foissner & Berger, 1996), while amoeba and flagellates were treated as single groups. After removal of formalin from the preserved sub–samples, Eumetazoa invertebrates were subsequently extracted under a Nikon SMZ–U stereomicroscope (30x), identified to species level in most of the groups (Table S5.2) using identification keys (Rundle *et al.* 2002, Tachet *et al.* 2010) and counted. The length and width of all counted organisms (Protozoa and Eumetazoa invertebrates) was measured to the nearest micrometre. Body dimensions of all counted Protozoa and meiofauna (Eumetazoa invertebrates whose body size is into the range of 0.45–500.00 µm) were transformed to biovolume after Reiss and Schmid–Araya (2010). Protozoa individual biovolume was directly converted to dry carbon content assuming 0.14 pg C/µm^3 (Putt & Stoecker 1996). For meiofauna individual biovolume was first converted into fresh mass using published gravity values (Feller & Warwik, 1998) following the approach of previous studies (i.e. Reiss & Schmid–Araya 2008, Tod & Schmid–Araya 2009, Peralta–Maraver *et al.* 2018a). Measurements of macroinvertebrates (Eumetazoa invertebrates whose body size is larger than 500.00 µm) were converted to dry mass using published body length and biovolume formula (Feller & Warwick 1998, Benke *et al.* 1999, Reiss & Schmid–Araya 2008, Tod & Schmid–Araya 2009). The individual carbon content of all Eumetazoa invertebrates was then calculated by using dry/wet mass ratio of 0.25 and dry mass/carbon content of 0.4 (Feller & Warwick 1998). Lastly, biomass (mg C/L) of all identified taxa was obtained

by multiplying carbon content with individual density (ind/L).

Following (Thompson *et al.* 2017), estimates of Protozoa and Eumetazoa invertebrates' α–diversity were made by setting a base sample size and using rarefaction based on Hill numbers. This approach is a robust method of comparing diversity values between communities when sample sizes differ (Chao *et al.* 2014). Furthermore, it solved related collinearity problems between diversity and biomass (as it occurs with other diversity indices, i.e. Shannon–Wienner diversity index). In this manner, both variables could be incorporated in the analytical models (see below). Calculations of α–diversity were made using the R package *iNEXT* (R Core Team 2018, Hsieh et al. 2014).

3.3 | *Biomass, functional diversity and potential metabolic activity of Prokaryota*

Prokaryotic biomass was assessed after cell counting in the filtered stored water. To stain the DNA of living cells, 200 µL of PicoGreen dye solution (Quant–iT™ PicoGreen™ dsDNA Assay Kit, Sigma–Aldrich) was added to 1 mL filtered water and incubated at 4°C for 15 min. Prokaryotic cell counts were then measured using an Accuri C6 flow cytometer (BD Biosciences) on slow with a forward scatter–H of 8000 and a side scatter–H of 2000. The list of individual events returned by the flow cytometer was extracted using the R packages *flowCore* and *flowViz* (Sarkar *et al.* 2008, Ellis *et al.* 2009, R Core Team 2018). Next, following Schaum *et al.* (2017) individual cell sizes were adjusted from the forward scatter values, and biomass values were inferred from published relationships between cell size and carbon content (Watson *et al.* 1977, Fuhrman and Azam 1980).

Prokaryotic potential activity (as aerobic metabolic potential to utilize different carbon sources) was measured by incubating filtered water sub–samples in BiOLOG

EcoPlate systems (Biolog Inc.). EcoPlates had 96 wells containing 31 different dissolved carbon sources and a blank (a control well which contains only water), replicated three times. EcoPlate substrates were grouped into six categories according to Feigl *et al.* (2017): carbohydrates, carboxilic acids, phenolic compounds, amino acids and polymers (grouping of substrates is available in Table S5.3). Each well also contained tetrazolium violet redox dye, which when reduced as consequence of prokaryotic respiration, varied from transparent to purple. A100–µL aliquot was pipetted into each EcoPlate well and incubated in the dark at 15 °C for 5 days (three replicates by zone and study site by EcoPlate). After this period, colour development of each carbon category was measured as optical density (*OD*) at 595 nm using a Biotek HT absorbance reader (Biotek, Swindon, U.K.). Following EcoPlate protocols, *OD* values were corrected by subtracting the blank values in each EcoPlate and setting negative values to 0. Corrected *OD* values were used to calculate the plate average well colour development (*AWCD*) as:

$$(1)\ AWCD = \sum OD_i / N$$

where OD_i is the corrected *OD* value of each substrate containing well and *N* is the number of substrates (31) (Gryta *et al.* 2014). The average well colour development values for each substrate (*Substrate AWCD*) were also obtained with the equation 2. For that propose, time OD_i represented the corrected *OD* value of the substrates within the substrate category and *N* was the number of substrates in the category (Kenarova *et al.* 2014). Finally, prokaryotic functional diversity was calculated as Shannon–Wiener diversity value (*H´*) based on substrate utilisation as:

$$(2)\ H' = -\sum_{i=1}^{Sr} P_i \ln(P_i)$$

where *Sr* is the number of wells with colour development and P_i is the proportional colour development of the well over total colour development of all wells of a plate.

3.4 | *Artificial substrata processing in the streambed sediments*

We measured rates of particulate organic carbon breakdown quantifying tea litter processing and loss of cotton tensile strength. Following Woodward *et al.* (2012), breakdown rates of green and red tea–leaves (mass loss), and cotton–strips (tensile–strength) were expressed as the exponential decay coefficient (k) in the formula

$$(3)\ X_t/X_0 = e^{-kt}$$

were X_0 was the initial leaf mass, or tensile strength and X_t was the value upon removal of the mesh–packages from the field at time t. The exponential coefficient t was expressed in terms of thermal sums (degree–days) in order to correct for potential temperature differences among streams and regions. Cotton–strips were accessible to all organisms in the assemblage that had the ability to penetrate into the mesh–packages. The tea–bag mesh size of 0.25 mm allowed microorganisms (as Prokaryota and Protozoa) to enter the tea–bags, but excluded Eumetazoan invertebrate groups. Thus, We could examine the role of different size groups of organisms in the decomposition rate of organic matter of streambed sediments.

Differences in lability between tea–bags mean that the standard Tea–bag Index (TBI) can be used to assess global litter decomposition rate (K) and long–term carbon stabilization coefficient (S). This technique has been shown to be very a reliable method in assessing litter processing in terrestrial and aquatic systems (Whigham *et al.* 2017, Djukic *et al.* 2018). A detailed explanation of the TBI is available in Keuskamp *et al.* (2013).

3.5 | *Statistical analysis*

First, differences in measured biological responses of biomass, α–diversity, functional diversity of Prokaryota and AWCD values between streambed compartments

(two levels factor: BZ and HZ) were assessed applying one–way ANOVA tests. Secondly, multiple linear regression techniques were used to build predictive models of the decay coefficients of green tea–bags (k_{green}), red tea–bags (k_{red}), cotton–strips (k_{cotton}), K and S depending on previous biological responses, streambed compartments and their interactions. Biomass was first $\log_{10}$ transformed to solve heterogeneity of the residuals in the ANOVA tests and the regression models, but this was not necessary for the rest of the responses. Continuous covariates in the regression models were first standardized by subtracting the mean and dividing by the standard deviation. Collinearity problems were detected among meiofauna and macroinvertebrates groups, among the different Protozoa groups and among plate AWCD and different substrates AWCD during data exploration. Therefore, plate AWCD was maintained as a potential covariate of the regression models, while substrates AWCD was not included. Intra–class correlation effects of the studied responses with the study site (samples collection from the same streams) and with catchment (streams sampled from the same catchment) were also detected during data exploration. Therefore, study site (30 levels) and catchment (10 levels) were incorporated in the ANOVA tests as random factors (two random factors ANOVA) and the regression models as random intercepts (Linear Mixed Models, LMMs). In addition, by adding previous random factors, environmental gradients (Table S5.1) could be removed as non–significant covariates from model equations during model selection routines (see below).

Statistical analyses were carried out using R software. Both ANOVA tests and LMMs were fitted using the restricted maximum likelihood estimation (REML) with the *lmer* function of the package lme4 (Djukic *et al.* 2016). In the case of LMMs, backward selection based on the Akaike Information Criterion (AIC) was applied to find the optimal models. Full models containing all variables and first–order interactions were

fitted first. Then, less influential variables (lower AIC) were dropped sequentially using the *drop1* function within R, and the reduced model was refitted at each step. Validation of underlying assumptions of normality and homoscedasticity in ANOVA tests and optimal LMMs residuals was applied following Zuur *et al.* (2009). The *anova* function (based on *F*–statistic) and the *summary* function (based on *t*–statistic) from the package lmerTest (Kuznetsova *et al.* 2015) were used to assess significance of ANOVA tests and LMMs coefficients respectively. These functions implement the Kenward–Roger's method for approximating degrees of freedom. This is a robust method, which allows obtaining the p–values related with the *F* and *t* statistic when applying mixed effect models (Kuznetsova *et al.* 2017). Finally, the marginal–R^2 and conditional–R^2 were calculated to assess model fit using the function *rsquaredGLMM* of the R package MuMIn (Nakagawa & Schielzeth 2013). The marginal–R^2 is concerned with variance explained by fixed factors, while the conditional–R^2 with variance explained by both fixed and random factors (Nakagawa & Schielzeth 2013).

4 | Results

4.1 | *Compartmentalization of the biological features in the streambed bioreactor.*

A total of 7136 Eumetazoa invertebrates, 11436 ciliates, and 2544 flagellates were collected and identified to measure biomass and α–diversity giving a good representation of the streambed assemblages. With the exception of Prokaryota biomass, all biological responses showed a marked decline within the HZ in comparison with the BZ (ANOVA tables including coefficients, degrees of freedom, *F*–statistic and p–values are available as Table S5.4). This pattern was highly significant for biomass and α–diversity of Eumetazoa invertebrates and Protozoa (Fig 5.2a,b), demonstrating a great

reduction and simplification of these assemblages in the HZ. Carbon substrate utilization, assessed via Biolog EcoPlates, showed that streambed compartments also differed significantly in the functional diversity and potential activity of the prokaryotic assemblage. Even though biomass of Prokaryota did not show any clear differences between compartments, the lower functional diversity values illustrated the simplification in the prokariotic assemblage within the HZ (Fig 5.2c). Furthermore, samples from the BZ had the highest Plate AWCD values, which were also highly significant (Fig. 5.2d). This trend was repeated for all Substrate AWCD (Fig 5.2e), implying that the prokaryotic assemblage in the upper streambed layer had a stronger metabolic potential to degrade the range of carbon sources provided.

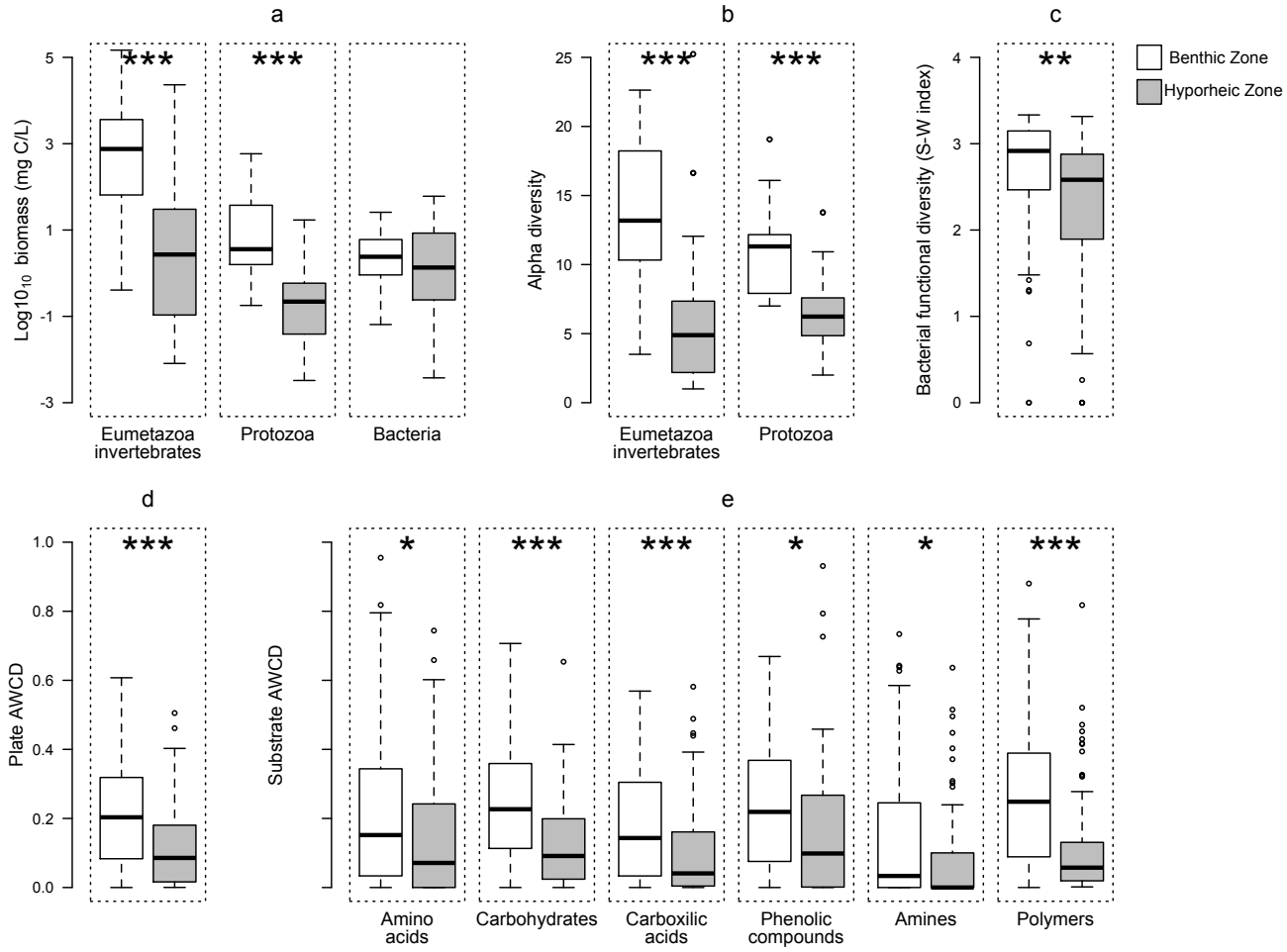

Fig 5.2. Descriptive Box–Plot showing the differences between the benthic and hyporheic zone for (a) biomass of the different studied groups, (b) α–diversity of Eumetazoa and Protozoa, (c) prokaryotic functional diversity, (d) Plate AWCD, and (e) Substrates AWCD. The bottom and top of the box represent the first and third quartiles of the data, the thick band inside represent the second quartile (median) of the data, the ends of the whiskers represent the minimum and maximum of all of the series of data, and dots represent atypical values (out–layers). Asterisks

4.2. | *Predictive modelling of substrata processing in the streambed*

After model selection routines and verification of model assumptions, we were able to build suitable predictive models for decay coefficients of cotton–strips (k_{cotton}), green tea–bags (k_{green}), red tea–bags (k_{red}) and for the long–term carbon stabilization coefficient (S, obtained after applying TBI) (Model equations are available in Table 1).

Table 5.1: Linear Mixed Models equations for decay coefficients of cotton–strips ($\boldsymbol{k_{cotton}}$), green–tea (k_{green}), red–tea (k_{red}) and long–term carbon stabilization coefficient (S). Predictive variables are: β_0 (fixed intercept), a_{site} and $a_{catchment}$ (random intercepts), *Zone* (factor with two levels: *BZ* and *HZ*), *Inv. Biomass* (biomass of Eumetazoa invertebrates), *Prot. Biomass* (biomass of Protozoa), *Bac. Biomass* (biomass of Prokaryota), *Inv.α–diversity* (α–diversity *of* Eumetazoa invertebrates) and *Prok.Functional diversity* (functional diversity of Prokaryota). Models assume that responses, model residuals (ε), a_{site} and $a_{catchment}$ follow a Gaussian distribution.

Response	Model equation
k_{cotton}	$\beta_0 + a_{\text{site}} + a_{catchment} + Zone \times \text{Log}_{10}(Inv.\ Biomass) + Zone \times Inv.\alpha\text{–}diversity + \varepsilon$
k_{green}	$\beta_0 + a_{\text{site}} + a_{catchment} + Zone + \text{Log}_{10}(Prot.\ Biomass) + Zone \times Inv.\alpha\text{–}diversity + \varepsilon$
k_{red}	$\beta_0 + a_{\text{site}} + a_{catchment} + Zone + \text{Log}_{10}(Prot.\ Biomass) + \text{Log}_{10}(Bac.\ Biomass) + \varepsilon$
S	$\beta_0 + a_{\text{site}} + a_{catchment} + Zone + \text{Log}_{10}(Inv.\ Biomass) + \text{Log}_{10}(Prot.\ Biomass) + \text{Log}_{10}(Prok.Functional\ diversity) + \varepsilon$

In contrast, global leaf litter decay coefficient (K, obtained after applying TBI) did not show any clear relationship with the streambed compartment (Fig S5.2) or the biological covariates, neither was it possible to propose an appropriate predictive model for this response. Cotton–strips showed the higher decay rate [Mean (SD) = 4.0 (3.0)$\times 10^3$], closely followed by green–tea leaves [Mean (SD) = 3.0 (0.6) $\times 10^3$], and finally rooibos tea [Mean (SD) = 0.7 (0.1)$\times 10^3$] as the more recalcitrant substratum. In

all models, the response depends on the fixed intercept (β_0), two random intercepts (a_{site} and $a_{catchment}$) and *Zone* (factor with two levels: *BZ* and *HZ*).

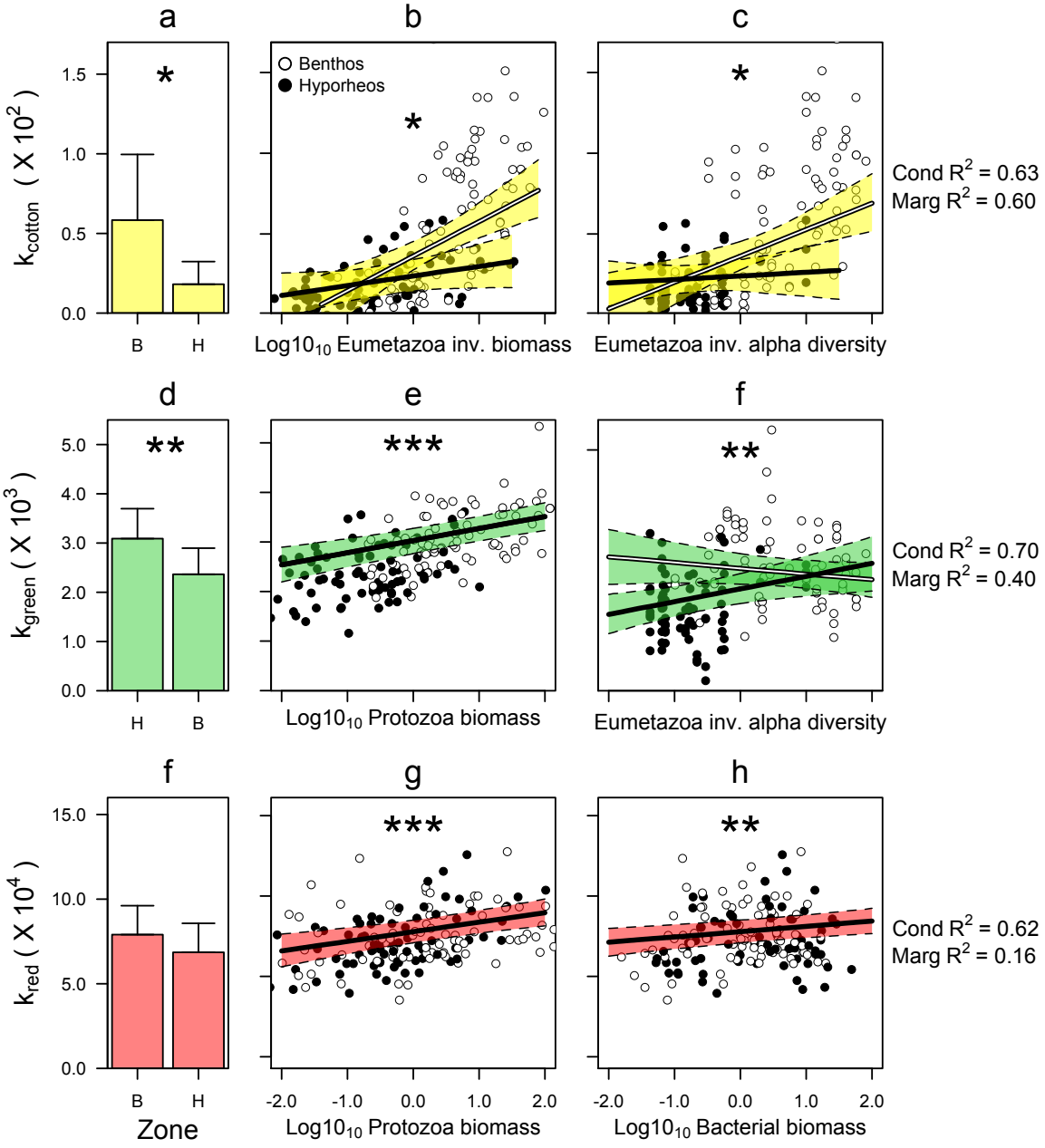

Fig 5.3. Multiple linear mixed regression models for the decay coefficients (responses) of cotton–strips (k_{cotton}; three top panels coloured in yellow), green tea–bags (k_{green}; three middle panels coloured in green), red tea–bags (k_{red}; three bottom panels coloured in red). White dots represent those values from the benthic assemblage (benthos), while black dots those from the hyporheic assemblage (hyporheos). Bar–plots on the left show the mean value (± SD) of each decay coefficient in the benthic (B) and the hyporheic (H) zone (two levels factor). Coloured shaded areas on the regression lines represent the 95%CI of the continuous covariates. Conditional R2 (Cond R^2) and the marginal R2 (Marg R^2) obtained for each model is attached on the right side of every three panels. Asterisks indicate significant effect of the coefficients in the models for the *t* statistic (*** $p < 0.0001$; ** $p < 0.001$; * $p < 0.05$). Note that plot shows standardized covariates.

Model for k_{cotton} included biomass (Log_{10}) and α–diversity of Eumetazoa invertebrates, the interaction between *Zone* and biomass of Eumetazoa invertebrates, and the interaction between *Zone* and α–diversity of Eumetazoa invertebrates. (**2**) Model for k_{green} included biomass (Log_{10}) of Protozoa and the interaction between *Zone* and α–diversity of Eumetazoa invertebrates. (**3**) Model for k_{red} included biomass of Protozoa and biomass (Log_{10}) of Prokaryota. (**4**) Model for S included biomass of Eumetazoa invertebrates and Protozoa, and functional diversity (S–W) of Prokaryota. Summary tables including coefficients, degrees of freedom, t–statistic and p–values are available in Table S5.5.

We observed a general pattern of reduction in responses k_{cotton}, k_{green} and k_{red} within the HZ compared to the BZ (Fig 5.3a,d and g). This reduction was significant for k_{cotton} and k_{green}, while k_{red} model did not detect any significant difference between streambed compartments. Accordingly, the BZ exhibited higher breakdown efficiency, and this was especially true for more labile substrata. The covariates of biomass and α–diversity of Eumetazoa invertebrates had a significant and positive effect on k_{cotton}. However, the interaction terms in k_{cotton} model showed a steeper positive effect of these covariates in the BZ than in the HZ (Fig 5.2b and c). Moreover, biomass of Protozoa had a highly significant boosting effect on k_{green} and k_{red} (Fig 5.3e, and g), independently of the streambed compartment. In contrast, even though α–diversity of Eumetazoa invertebrates was maintained as an effective predictor in k_{green} model, this variable did not have a significant effect on the response itself. It depended on the interaction with *Zone*, which revealed the significant positive effect of α–diversity of Eumetazoa invertebrates on k_{green} within the HZ (Fig 5.3f). The relationship between α–diversity of Eumetazoa invertebrates and k_{green} remained less clear (or even slightly negative) in the

BZ. Lastly, k_{red} model also detected the significant positive effect of prokaryotic biomass on the response (Fig 5.3i).

Finally, all the predictive variables included in *S* model had a significant effect on the response. In this case, *S* reached higher values in the HZ than in the BZ (Fig 5.4a). Furthermore, the rest of the covariates showed a negative relationship with *S* (Fig 5.4b,c and d), especially for biomass of Protozoa and Eumetazoa invertebrates. Therefore, the inhibiting effect of the environment on decomposition of labile fraction of litter was reduced in the upper streambed compartment resulting from the higher biomass and functional diversity of Prokaryota (lower *S*).

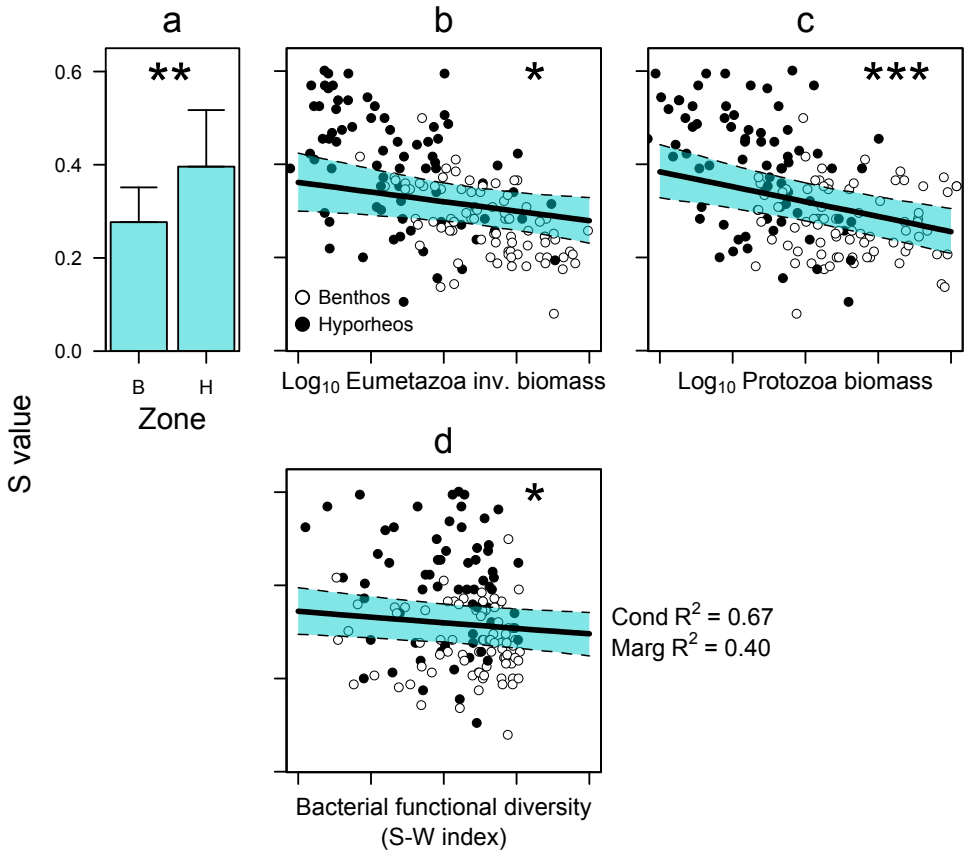

Fig 5.4. Multiple linear mixed regression model for the long–term carbon stabilization factor (S, response). White dots represent those values from the benthic assemblage (benthos), while black dots those from the hyporheic assemblage (hyporheos). Bar–plot shows the mean value (± SD) of S in the benthic (B) and the hyporheic (H) zone (two levels factor). Coloured shaded areas on the regression lines represent the 95%CI of the continuous covariates. Conditional R2 (Cond R2) and the marginal R2 (Marg R2) obtained for each model is attached on the right side of panel d. Asterisks indicate significant effect of the coefficients in the models for the t statistic (*** $p < 0.0001$; ** $p < 0.001$; * $p < 0.05$). Note that plot shows standardized covariates.

5 | Discussion

5.1 | *Compartmentalization of the biological features in the streambed bioreactor.*

The dramatic reduction in biomass and α–diversity of Protozoa and Eumetazoa invertebrates within the HZ in this study is in agreement with the widely reported pattern of simplification of these assemblages with increasing depth in the streambed (i.e. Schmid–Araya 1994, Sliva & Williams 2005, Andrushchyshyn *et al.* 2007, Reynolds & Benke 2012, Peralta–Maraver et al 2018b, Dunscombe et al, 2018). This likely results from the reduction in oxygen availability and pore space due to sediment agglomeration, which limits the vertical distribution of all organisms, but especially those with larger body size (Schmid–Araya 1994, Maridet & Philippe 1995, Strayer *et al.* 1997; Stead *et al.* 2004, Peralta–Maraver *et al.* 2018b). Prokaryotic biomass, however, did not show any clear difference between compartments in contrast to several previous studies, which found that abundance and productivity of Prokaryota peaked in the upper layer of the streambed sediments (i.e. Fischer et al 2002a, Fischer et al 2002b, Huang *et al.* 2011, Navel *et al.* 2011). However, biofilms are firmly attached to the substrata and our sample processing method may not have detached sufficient biofilm to detect differences between compartments. Nevertheless the significant reduction in prokaryotic functional diversity confirmed that the general pattern of simplification of the streambed assemblage within the HZ compared to the BZ is also true for this group. Furthermore, the potential metabolic activity of prokaryote consortia (measured as AWCD) was notably greater in the BZ for all the dissolved organic carbon sources. Previous laboratory mesocosm experiments have shown that activity of Prokaryota consuming dissolved organic carbon (including low–molecular weigh carbon substances and polysaccharides) decreases sharply with depth in the streambed (Fischer et al

2002a, Foulquier *et al.* 2011). However, it is possible that lower availability of the resource with increasing depth might explain part of this response. In this study we measured prokaryotic metabolic activity in the same conditions of dissolved organic carbon availability (Biolog EcoPlates), and our results demonstrate unequivocally the lower metabolic capability of hyporheic prokaryotic consortiums in streambed systems.

5.2 | *Hypothesis 1: The top layers are the most active part of the streambed bioreactor and therefore leaf litter processing is expected to be higher in the BZ than in the HZ*

Our inferential models mainly upheld the predicted reduction in leaf litter breakdown in the HZ, particularly for the decay coefficients of labile compounds (k_{cotton} and k_{green}). Results from our regional–scale approach are in agreement with previous experimental assessments (Cornut *et al.* 2010) and local–scale survey studies (i.e. Smith & Lake 1993, Naamane *et al.* 1999), which documented that leaf litter processing is generally depressed in the HZ. Likewise, our findings reinforce the suggestion that the BZ is the key site of biochemical turnover in the streambed bioreactor (Knapp *et al.* 2017). Our study (and the studies cited above) encompasses permanent streams and rivers. Using a very similar analytical and sampling methodology, Burrows *et al.* (2017) reported contrasting results in a large survey study in subtropical intermittent streams of Eastern Australia. These authors showed that decay coefficients of leaf litter and cotton–strips were disproportionally greater in the HZ (at 30 cm depth) than in the surface. Intermittency in surface flows an extreme disturbance for sediment organisms (Robertson *et al.* 1995, Townsend 1997, Robertson 2000) and part of the BZ biota may take refuge in the HZ, where humid conditions remain after surface drying (Robertson and Wood 2010, Febria et al 2012). As a consequence, biomass, α–diversity and

microbial functional diversity and thus leaf litter processing should increase in the HZ of intermittent streams.

Our inferential model for *S* coefficient predicted that a significantly higher proportion of leaf litter escapes from processing in the HZ and becomes recalcitrant. This is especially relevant in the context of the global carbon cycle. It has been argued that the streambed might have a significant role both in the mineralization and sequestration of organic carbon, and that integrating these fluxes into the traditional carbon cycle is needed for appropriate CO_2 management and climate change mitigation (Battin *et al.* 2009). Our findings highlight these fluxes, but also emphasise the importance of understanding the contrasting role of the two compartments in the streambed. Mineralization clearly becomes more important in the BZ, while the HZ fulfils the role of an organic carbon sink by reducing CO_2 emissions from rivers.

The reduction of decay coefficient within the HZ was also observed for the rooibos tea (k_{red}), but differences between compartments were too weak to be detected as significant by our models. Furthermore, the regression model explained only a small part of the observed variation (very low marginal R^2 and conditional R^2), probably because of the recalcitrant nature of rooibos tea as reported by Keuskamp *et al.* (2013). In the case of *K*, it was not even possible to infer any tendency between compartments or the rest of the covariates, probably because of the large amount of variation in the values. It is possible that our incubation period was too long (even thought the standard used in soils systems is 90 days), because breakdown in our aquatic systems was rapid. Hence, rooibos tea could have entered in the second phase of decomposition (breakdown of recalcitrant fraction), resulting in an increase of noise in the calculated *K* but not *S* (see stepwise TBI protocols). Similarly, Whigham et al (2017) compared leaf litter decay coefficients in the riparian soil of two contrasting wetlands systems using

the TBI with a 1 year incubation period. They were also unable to detect differences in *K* between treatments (presence or absence of alder in the system), while it was possible for *S*.

5.3 | *Hypothesis 2: The whole assemblage of organisms is involved during streambed leaf litter processing.*

Our inferential models support our second hypothesis. Both the increases in biomass of Eumetazoa invertebrates and α–diversity stimulated the rate of leaf litter breakdown and reduced the proportion of sequestered labile organic carbon in the streambed. The positive effect of biomass and α–diversity of Eumetazoa invertebrates on the rate in which leaf litter is processed has been previously reported in a number of studies (i.e. Whigham & Malmqvist 2000, McKie *et al.* 2008). In contrast, its relationship with the stabilisation of labile carbon from leaf litter has not yet been assessed. During leaf litter breakdown, a proportion of the labile compounds stabilises and becomes recalcitrant (Prescott 2010) as a consequence of environmental factors (i.e. reacting with dissolved cations; Berg & Meentemeyer 2002). Our results suggest that the life activities of Eumetazoa invertebrates act in opposition to the environmental influence on organic matter retention in the streambed. In the case of k_{cotton}, it is reasonable to advocate for direct consumption of the substratum (cotton–strips were fully accessible for Eumetazoa invertebrates). Furthermore, cotton–strips were a rapidly processed substratum, which could mean that extensive microbial conditioning prior to consumption by Eumetazoa invertebrates is unnecessary (Golladay *et al.* 1983, Gonçalves *et al.* 2017). On the other hand tea–bags were not directly consumed by Eumetazoa invertebrates and therefore, other processes gained higher importance behind the outcome for k_{green} and *S*. The group of Eumetazoa invertebrates comprise a variety of organisms, which differ in biological traits such as body size, body shape,

functional traits or adaptations to movement. These diverse assemblages might undertake a variety of non-trophic engineering phenomena, such as bioturbation and bioirrigation in the sediments (Usio & Townsend 2004, Baranov *et al.* 2016a, Baranov *et al.* 2016b) creating an environment of high biochemical transformation and leaf litter processing.

Models for k_{green}, k_{red} and S revealed that an increase of biomass of Protozoa also stimulated the rate of leaf litter breakdown and reduced the proportion of sequestered labile compounds. This is the first field study to demonstrate the involvement of Protozoa in leaf litter processing and it supports preliminary laboratory results (Ribblett *et al.* 2005). Several potential mechanisms may explain the effect of protozoan biomass on k_{green}, k_{red} and S. Constant grazing on biofilms by Protozoa keeps Prokaryota consortia in the active log–phase of growth (Fenchel & Jørgensen 1977) and they usually selectively consume less active Prokaryota (Shapiro *et al.* 2010). Thus leaf litter breakdown rates may increase even though the total abundance of Prokaryota decreases as a consequence of protozoan grazing (Ribblett *et al.* 2005). Additionally, Protozoa produce waste products that fuel the metabolism of Prokaryota (Jansson *et al.* 1999), induce the recycling of nutrients (Shapiro *et al.* 2010) and increase the absorption surface of the biofilms after grazing (Peralta–Maraver *et al.* 2018a). However, controlled experiments are still necessary to test previous proposed mechanisms and completely elucidate how protozoa contribute to this ecosystem processes.

The relationship of Prokaryota with leaf litter processing was less obvious than for eumetazoan invertebrates and Protozoa but we can infer from our model for k_{red} that breakdown associated with microbial biomass is most important during the processing of recalcitrant leaf litter material. Contrary, previous studies have suggested that the contribution of Prokaryota to leaf litter breakdown could be higher than inferred from

biomass (Findlay and Arsuffi 1989, Baldy and Gessner 1997). Our models for k_{red} and S detected prokaryote biomass and functional diversity as drivers in this process agreeing with previous research (Battin *et al.* 2016). Leaf litter is mainly composed of cellulose, but also other carbon sources such as lignin, hemicellulose and pectin (Yadav and Malanson, 2007), requiring numerous enzymatic routes for degradation (Schneider *et al.* 2012). Accordingly, high functional diversity allowing the consumption of different carbon sources results in a large potential for facilitative interactions and resource partitioning (Gessner *et al.* 2010). Our findings state that diverse functional assemblages of Prokaryota improve the efficiency of the streambed bioreactor in processing complex litter compounds and thus also reduce the environmental sequestration of labile carbon.

6 | Conclusion

We reported that, under permanent flow conditions, the BZ is most important in the functioning of the streambed bioreactor during leaf litter processing, while the HZ has a main role in the sequestration and storage of organic carbon. Furthermore, our study is the first to apply the TBI in the streambed, and our expertise using the TBI could be very useful to adjust incubation periods in next studies in freshwater systems. Our results demonstrate that the bioreactor ability of the streambed is sustained and maintained by diverse and active assemblages. We showed that all the groups intervene (directly or indirectly) in this process and highlight the necessity of include them in future research of streambed functioning. Besides, the inferential models we presented are a suitable tool in future studies to predict the efficiency of streambed systems in the processing of leaf litter, based on the biological features of the assemblage and the difference between the BZ and the HZ. Although the benthos is more efficient than the hyporheos in the processing of complex organic carbon sources that require a sequential

breakdown process (leaf litter here), this might not be true for other nutrient sources or contaminants dissolved in the water (also with higher bioavailability). The HZ is indeed largely recognised as being important in the attenuation of dissolved nutrient and contaminant in freshwater systems (i.e. Lewandowski *et al.* 2011, Peralta–Maraver *et al.* 2018a). However, the complex mechanisms by which the organisms drive the leaf litter processing and the relative importance of them (i.e. bioturbation *vs* grazing) remain to be elucidated.

7 | Appendix chapter 5

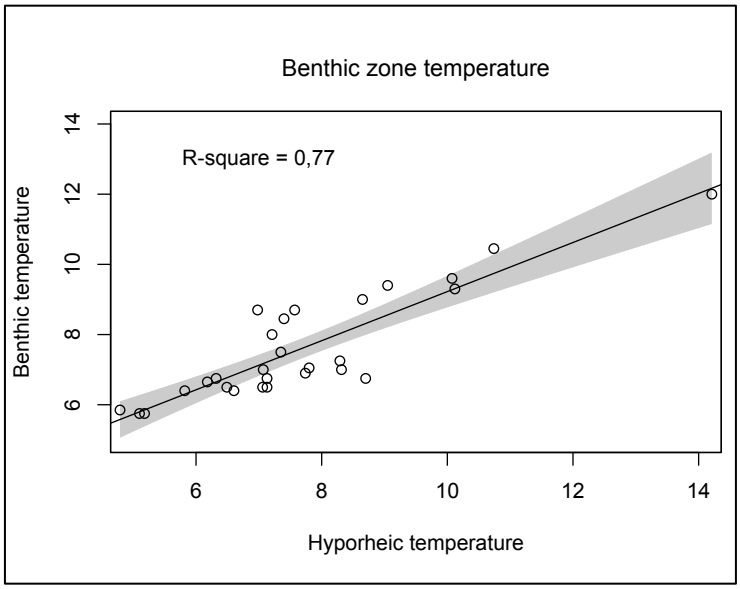

Fig S5.1. Linear regression model predicting benthic temperature as a response of the hyporheic temperature (covariate). β_0: intercept, β_1: slope, R-square: coefficient of determination.

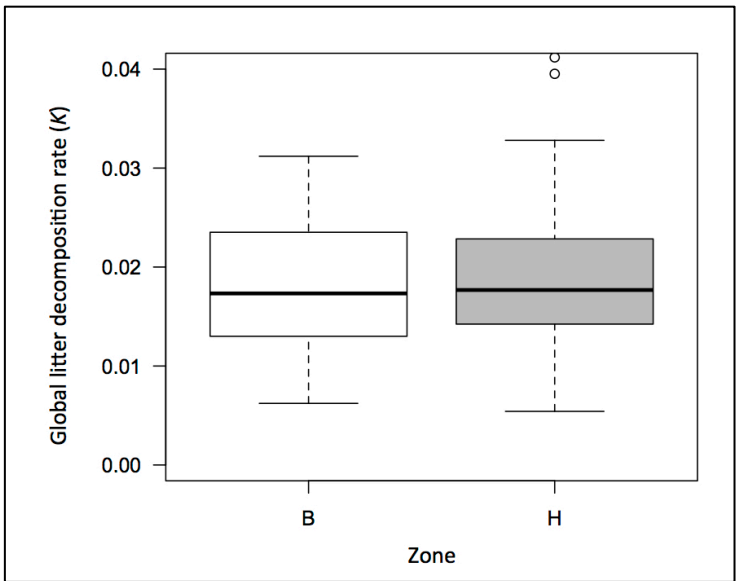

Fig S5.2. Descriptive Box-Plot showing global litter decay rate (K) values in the benthic and hyporheic zone of the studied systems.

Table S5.1. Incubation period of the artificial substratum and physicochemical characteristics of the studied streams. It has also included the adjacent land-use of the study sites (WO = Woodland; PA = Pasture; AR = Arable; RO = Road; PL = Parkland; WL = Woodland; SA = Set aside; WE = Wetland; CO = Conifer plantation; UR = Urban; GR = Grassland). N: nitrate (mg/L), P: phosphate (mg/L), DOM: dissolved organic carbon (mg/L).

Stream	Catchment	Altitude (m)	Incubation (days)	pH	N	P	DOM	Land use
Bure	Bure	20,00	31,00	7,93	6,23	0,06	10,32	WO
Howe Beck	Eden	168,99	49,00	8,00	0,67	0,01	13,35	PA
Leith	Eden	108,99	49,00	8,33	2,21	0,05	--	PA
Lyvennet	Eden	109,99	49,00	8,57	1,11	0,02	11,60	PA
Moreland Beck	Eden	115,00	49,00	8,40	1,54	0,02	11,95	PA
Crowdundle	Eden	103,00	45,00	7,98	0,89	0,01	11,43	AR, RO
Glaven	Glaven	14,00	31,00	8,11	6,75	0,29	10,61	WO
Broadstone	Medway	71,00	29,00	5,50	3,33	0,31	9,61	WO
Lone Oak	Medway	118,99	29,00	6,40	0,55	0,03	9,77	WO
Old Lodge	Medway	124,99	29,00	6,60	0,55	0,03	9,77	PL, WL
Stiffkey	Stiffkey	10,00	31,00	7,80	7,00	0,13	10,61	SA
Anton	Test	44,00	45,00	8,18	8,11	0,03	11,43	WE
Test	Test	43,00	45,00	8,08	8,17	0,05	10,68	WE
Beverley Brook	Thames	11,00	30,00	7,56	18,60	0,34	7,32	PL
Kennet	Thames	124,99	45,00	7,72	7,51	0,03	10,05	SA
Loddon	Thames	60,00	45,00	7,61	12,04	0,17	8,51	PA, WL
Lyde	Thames	60,00	45,00	8,19	7,03	0,01	10,17	PA, AR
GI1	Tywi	190,99	43,00	5,90	0,23	0,01	--	PA
LI3	Tywi	354,98	43,00	6,12	0,48	0,00	--	CO
LI6	Tywi	294,99	43,00	6,67	0,04	0,00	--	PA
LI7	Tywi	340,98	43,00	6,89	0,03	0,01	--	PA
LI8	Tywi	316,99	43,00	5,23	0,30	0,01	--	CO, PA
Waveney	Waveney	10,00	61,00	7,54	6,11	0,09	10,17	PL
Tat	Wensum	47,00	33,00	7,42	8,29	0,07	9,49	WO
Wensum	Wensum	29,00	33,00	7,24	6,12	0,06	9,81	PA
Deadwater	Wey	76,00	29,00	7,68	0,88	0,03	9,08	WO
Lamports	Wey	99,99	29,00	7,31	4,58	0,05	9,71	UR
Nadder	Wey	111,99	29,00	7,76	9,30	0,27	8,54	PL
Oakhanger	Wey	79,00	29,00	7,58	2,09	0,18	9,51	PA
Wey	Wey	44,00	29,00	7,77	4,61	0,15	9,63	WO, GR

Table S5.2. Identified Taxa list in the benthic (B) and hyporheic (H) zones for the 30 studied streams during the study period from October 2016 to December 2016: Anton (Ant), Beverly brooks (B.br), Broadstone (Bro), Bure (Bur), Crowdudle (Cro), Deathwater (Dea), GI1, Glaven (Gla), Howe beck (How), Kennet (Ken), Lamports (Lam), Leith (Lei), LI3, LI6, LI7, LI8, Loan oak (Loa), Loddon (Lod), Lyde (Lyd), Lyvennet (Lyv), Morland beck (Mor), Nadder (Nad), Oak.hanger (Oak), Old lodge (Old), Stiffkey (Sti), Tatt (Tat), Test (Tes), Waveney (Wav), Wennsum (Wen), Wey. Taxonomic resolution = species (.sp), tribu (Tr.), sub-family (Sf.), family (F.), order (O.), sub-class (Sc.).

Taxa	Zone	Stream
EUMETAZOA *INVERTEBRATES*		
Non-insects		
Nematoda	B/H	Ant, B.br, Boa, Cro, Dea, GI1, Gla, How, Ken, Lam, Lei, LI3, LI6, LI7, Lod, Lyd, Lyv, Mor, Nad, Oak, Sti, Tat, Tes, Wav, Wen, Wey
Acari	B	Ant, Det, Lam, Lyv, Wen
Asellus sp.	B	B.br, Dea, Gla, Lam, Lei, Tes, Wav
Gammarus sp.	B/H	Ant, B.br, Bur, Cro, Dea, Gla, How, Ken, Lam, Lei, LI7, Lod, Lyd, Lyv, Mor, Nad, Sti, Tat, Tes, Wav, Wen, Wey
Gastotricha	B	B.br
Sc. Oligochaeta	B/H	Ant, B.br, Bro, Bur, Crow, Dea, GI1, Gla, How, Ken, Lam, Lei, LI3, LI6, LI7, LI8, Loa, Lod, Lyd, Lyv, Mor, Nad, Oak, Old, Sti, Tat, Tes, Wav, Wen, Wey
Cl. Ostracoda	B/H	Ant, Bro, Lam, LI8, Lod, Lyd, Old, Tat, Tes, Wen
Sphaerium sp	B	Lod
Corophium sp.	B	Lei, Wen
Sc. Hirudinea	B/H	Ant, B.br, Cro, Gla, How, Ken, Lei, Lod, Mor, Sti, Tes
O. Harpacticoida	B/H	Ant, B.br, Gla, Ken, Lam, Lei, Loa, Lyd, Lyv, Mor
Insects (O. Odonata)		
Calopterix sp.	B	B.br

Continue table S5.2

Insects (O. Plecoptera)		
Amphinemura sp.	B	Cro, LI7
Isoperla sp.	B/H	Ant, Cro, Dea, GI1, How, Ken, LI·, LI6, LI7, LI8, Loa, Lyv, Mor, Old, Sti
Leuctra sp.	B	Cro, GI1, LI3, LI7, LI8, Loa, Lyv
Insects (O. Ephemeroptera)		
Baetis sp.	B	Ant, Bur, How, Ken, Lei, Lyd
Caenis sp.	B	Gla, Kenn, Lyv, Wav, Wey
Ephemerella sp.	B	Ken, Lam
Paraleptophlebia sp.	B	How, Mor
Insects (O. Trichoptera)		
F. Leptoceridae	B	Ant
Hydropsyche sp.	B	Ant, GI1, Lud, Lyv, Mor, Wav, Wen, Wey
Hyporyacophila sp.	B	Lam
Ithytrichia sp.	B	Ant, Bur, Lyd, Lyv, Sti, Tes, Wen
Lepidostoma sp.	B	Gla, Sti, Wen
F. Leptoceridae	B	Lam
Plectronemia sp.	B	LI3
Polycentropus sp.	B	Sti
Prosorhyacophila sp.	B	Lyd
Rhyacophila sp.	B	GI1, How, Ken, Mor
Nemotaulius sp.	B	Tes
Sericostoma sp.	B	Ant, GI1, How, Lyv, Tes, Wav, Wen
Sf. Agapetinae	B	Lyd
Sf. Pseudoneuroclipsis	B	Lyd
Wormaldia sp.	B	Ken
Silo sp.	B	Ant
Oecetis sp.	B	Tes
Insects (O. Diptera)		
Chironomidae pupae	B	Dea, Ken, Nad, Wav, Wen
Sf. Ceratopogoninae	B	Ant, Bro, Gla, How, Lam, Lei, LI3, LI8, Lyv, Stif, Tat, Tes, Wey
Sf. Clinocerinae	B	Ant, GI1, Lei, Lyv
Sf. Hemerodromiinae	B	Bur, GI1, Lam, Lyd
Sf. Orthocladinae	B/H	Ant, B.br, Bro, Bur, Bur, Cro, Dea, GI1, Gla, How, Ken, Lam, Lei, LI·, LI6, LI7, LI8, Loa, Lod, Lyd, Lyv, Mor, Nad, Old, Sti, Tat, Tes, Wav, Wen, Wey

Continue table S5.2

Sf. Tanypodinae	B/H	Ant, B.br, Bro, Cro, Dea, GI1, Gla, How, Ken, Lam, Lei, LI3, Loa, Lod, Lyd, Lyv, Mor, Oak, Old, Sti, Tes, Wav, Wen
F. Stratyomiidae	B	Cro, Lyv
Tr. Chironomini	B/H	Ant, B.br, Bor, Bur, Cro, Dea, GI1, Gla, How, Ken, Lei, LI3, LI6, Loa, Lod, Lyd, Lyv, Mor, Nad, Oak, Sti, Tat, Nad, Wav, Wey
Tr. Limoniini	B	Cro, LI6, Mor, Tat, Tes, Wav
Tr. Pediciini	B/H	Gla, LI7, Lyd, Wey
Tr. Simuliini	B	GI1, Gla, How, Lei, Old, Wav, Wen
Tr. Tanytarsini	B/H	Ant, B.br, Bro, Bur, Cro, GI1, How, Ken, Lam, Lei, Lod, Lyd, Lyv, Mor, Nad, Old, Tat, Tes, Wav, Wen, Wey
F. Anthomyiidae	B	Lam
Tr. Hexatomini	B	LI7
Insects (O. Coleoptera)		
Elmis sp. Adult	B	Ant, Gla, Lyd
Elmis sp. Larve	B/H	Ant, Gla, Ken, Lam, Lei, Lod, Mor, Sti, Tes, Wen
Haliplus sp.	B	How, Ken, Lei, Lyv, Mor, Wav, Wey
Limnius sp. Adult	B	How, Sti
Limnius sp. Larve	B/H	Ant, Cro, GI1, Gla, Lei, Sti, Tes, Wav, Wen
Orectochilus sp. Larve	B	Lyv
Riolus sp larve	B	Lyv
CILIATES		
Sc. Cyrtophorida	B/H	Ant, B.br, Bro, Bur, Cro, Dea, How, Ken, Lam, LI6, LI8, Loa, Lod, Wen
Sc. Gymnostomatida	B/H	Ant, B.br, Bro, Bur, Cro, Dea, GI1, Gla, How, Ken, Lam, Lei, LI3, Li6, Li7, LI8, Loa, Lod, Lyd, Lyv, Mor, Nad, Oak, Old, Tes, Wav, Wen, Wey
Sc. Heterotrichia	B/H	Ant, B.br, Bro, Bur, Cro, Dea, GI1, Gla, How, Ken, Lam, Lei, LI3,LI6, LI7, Loa, Lod, Lyv, Mor, Nad, Oak, Old, Sti, Tat, Wav, Wen, Wey
Sc. Hymenostomata	B/H	Ant, B.br, Bro, Bur, Cro, Dea, GI1, Gla, How, Ken, Ken, Lam, Lei, LI3, LI6, LI7, LI8, Loa, Lod, Lyd, Luv, Mor, Nad, Oak, Old, Sti, Tat, Tes, Wav, Wen, Wey
Sc. Hypotrichia	B/H	Ant, B.br, Bro, Bur, Cro, Dea, GI1, Gla, How, Ken, Lam, Lei, LI3, LI6, LI7, LI8, Loa, Lod, Lyd, Lyv, Mor, Nad, Oak,Old, Stif, Tes, Wav, Wen, Wey

Continue table S5.2

Sc. Odontostomatida	B/H	B.br, Dea, Gla, Lod, Lyv, Old, Tat
Sc. Oligotrichia	B/H	Ant, Bro, Cro, LI3, LI6, LI7, Loa, Sti, Wen
Sc. Peritrichia	B/H	Bro, Bur, Cro, Dea, GI1, Gla, How, Ken, Lam, Lei, LI6, LI7, LI8, Loa, Lod, Lyd, Lyv, Mor, Nad, Oak, Old, Sti, Tat, Tes, Wen, Wey
Sc. Pleurostomatida	B/H	Ant, B.br, Bro, Bur, Cro, Dea, GI1, How, Ken, Lam , Lei, LI3, LI6, LI7, LI8, Loa, Lyd, Mor, Old, Stif, Tat, tes, Wav, Wen, Wey
Sc. Prostomatida	B/H	Ant, B.br, Bro, Bur, Cro, Dea, GI1, Gla, How, Ken, Lam, LI8, Loa, Lod, Mor, Nad, Old, Stif, Tes, Wen, Wey
Sc. Suctoria	B/H	Ant, B.br, Bro, Bur,C ro, Dea, Gla, How, Ken, Lam, Lei, LI3, LI6, LI7, LI8, Loa, Lod, Lyd, Lyv, Mor, Nad, Oak, Old, Tat, Tes, Wav, Wen, Wey
Cyst	B/H	Ant, B.br, Bro, Bur, Cro, Dea, Gla, How, Ken, Lam, Lei, LI8, Loa, Lod, Lyd, Lyv, Mor, Nad, Oak, Old, Tat, Wav, Wen, Wey
FLAGELATES		
Flagelates	B/H	Ant, B.br, Bro, Bur, Cro, Dea, GI1, Gla, How, Lod, Lyd, Luv, Mor, Nad, Oak, Old, Sti, Tat, Tes, Wav, Wen, Wey

Table S5.3. Carbon source categories grouping the BIOLOG EcoPlate substrates.

Carbon source category	**Eco-plate substrate**
Amino acids	L- arginine L-asparagine L-phenylalanine L-serine glycyl-L-glutamic acid L-theronine
Carbohydrates	D-mannitol, glucose-1-phosphate, D,L- alpha-glycerol phosphate, beta-methyl-D-glucoside, D-galactonic acid-gamma-lactone, i- erythritol, D-xylose, N-acetyl-D-glucosamine, D-cellobiose,

Continue table S5.3	
	alpha-D-lactose
Carboxylic acids	D-glucosaminic acid D-malic acid itaconic acid pyruvic acid methyl ester D- galactouronic acid alpha-ketobutiryc acid gamma-hydroxybutyric acid
Phenolic compound	2-hydroxy benzoic acid 4-hydroxy benzoic acid
Amines	phenylethylamine putrescine
Polymers	Tween 40 Tween 80 alpha- cyclodextrine glycogen

Table S5.4. ANOVA tables for the comparison of ecological variables (taxonomic group biomass, taxonomic α-diversity, bacterial functional diversity, EcoPlate AWCD and substrates AWCD) between benthic zone and hyporheic zone. Significance codes: 0 (***), 0.001 (**), 0.01 (*) and 0.05 (·). A Kenward-Roger approximation was used to calculate the effective degrees of freedom (DF).

	Sum Sq	DF	Den DF	F value	P (>F)	Sig
(1) Log_{10} eumetazoa invertebrates biomass						
Zone	191.49	1	130.56	202.55	< 0.001	***
(2) Log_{10} protozoa biomass						
Zone	93.50	1	131.26	168,09	< 0.001	***
Log_{10} bacterial biomass ~ zone						
Zone	1,56	1	130,79	2.79	0,096	.
(3) α-diversity eumetazoa invertebrates						
Zone	2687.10	1	129.22	257.13	< 0.001	***
(4) α-diversity protozoa						
Zone	640.12	1	131.17	131.07	< 0.001	***
(5) Bacterial functional diversity (S-W, EcoPlate)						
Zone	5.63	1	134.24	9.87	0,002	**

Continue table S5.4

(6) EcoPlate AWCD						
Zone	0.32	1	134.04	19.57	< 0.001	***
(7) Amino acids AWCD						
Zone	0,15	1	134.26	4,34	0,039	*
(8) Carbohydrates AWCD						
Zone	0,50	1	134.13	22,46	< 0.001	***
(9) Carboxylic acids AWCD						
Zone	0,23	1	134.78	11,51	0,001	***
(10) Phenolic compunds AWCD						
Zone	0,17	1	134.99	5,12	0,025	*
(11) Amines AWCD						
Zone	0,12	1	134.70	4,46	0,036	*
(12) Polymers AWCD						
Zone	0,79	1	133.75	25,92	< 0.001	***

Table S5.5. Summary tables of the fitted predictive models (LMM): Coefficients (*Coef*), standard errors (*SE*), degrees of freedom (*DF*), *t*-values and P-values (*P*). Significance codes (*Sig*): 0 (***), 0.001 (**), 0.01 (*) and 0.05 (·). A Satterthwaite approximation was used to calculate the effective degrees of freedom.

	Coef	SE	DF	t value	P (>|t|)	Sig
(1) Decay rate cotton-strips (k_{cotton})						
Intercept	0,00364	0,00045	20,66238	7,68492	0,00000	***
Zone (HZ)	-0,00124	0,00059	99,38039	-1,99538	0,04874	*
Log_{10} Biomass (Eumetazoa invertebrates)	0,00215	0,0005	144,73195	4,18834	0,00005	***
α-diversity (Eumetazoa invetrtebrates)	0,00164	0,00045	117,85807	3,47338	0,00072	***
Zone (HZ) : Log_{10} biomass (Eumetazoa invertebrates)	-0,00154	0,00059	145,15796	-2,56137	0,01145	*
Zone (HZ) : α-diversity (Eumetazoa invetrtebrates)	-0,00141	0,0006	119,68402	-2,28166	0,02428	*
(2) Decay rate green tea bags (k_{green})						
Intercept	0,00303	0,00013	15,74523	23,15971	0,00000	***
Zone (HZ)	-0,00034	0,00011	149,2007	-2,94196	0,00378	**
Log_{10} biomass (Protozoa)	0,00024	0,00005	148,65662	4,92143	0,00000	***
α-diversity (Eumetazoa invertebrates)	-0,0001	0,00007	150,21799	-1,29629	0,19686	
Zone (HZ) : α-diversity (Eumetazoa invertebrates)	0,00031	0,00009	145,98517	3,30264	0,00120	**

Continue table S5.5

(3) Decay rate red tea bags (k_{red})						
Intercept	0,00078	0,00004	11,93632	20,68177	0,00000	***
Zone (HZ)	-0,00003	0,00003	138,3786	-0,89598	0,37182	
Log_{10} biomass (Protozoa)	0,00006	0,00001	145,0219	3,85344	0,00017	***
Log_{10} biomass (Bacteria)	0,00003	0,00001	145,11873	2,70935	0,00755	**
(4) Decay rate cotton-strips (*S*)						
Intercept	0,31966	0,02091	13,87814	15,17215	0,00000	***
Zone (HZ)	0,05162	0,01911	145,0262	2,65097	0,00892	**
Log_{10} Biomass (Eumetazoa invertebrates)	-0,0205	0,00976	147,3874	-2,03786	0,04335	*
Log_{10} Biomass (Protozoa)	-0,03211	0,00832	139,71178	-3,7677	0,00024	***
Bacterial functional diversity (S-W index)	-0,01216	0,00608	136,19636	-1,97787	0,04996	*

8 | References

Abelho, M. Effects of leaf litter species on macroinvertebrate colonization during decomposition in a Portuguese stream. *Int. Rev Hydrobiol*. **93**, 358–371 (2008).

Andrushchyshyn, O.P. *et al.* Ciliate communities in shallow groundwater: seasonal and spatial characteristics. *Freshwater Biol.* **52**, 1745–1761 (2007).

Baranov, V. *et al.* Bioturbation enhances the aerobic respiration of lake sediments in warming lakes. *Biol. Lett.* **12**, 20160448 (2016a)

Baranov, V. *et al.* Effects of bioirrigation of non–biting midges (Diptera: Chironomidae) on lake sediment respiration. *Sci. Rep.* ***6***, 27329 (2016b).

Battin, T.J. *et al.* Biophysical controls on organic carbon fluxes in fluvial networks. *Nat. Geosci.* **1**, 95–100 (2008).

Battin, T.J. *et al.* The boundless carbon cycle. *Nat. Geosci.* **2**, 598 (2009).

Battin, T.J. *et al.* The ecology and biogeochemistry of stream biofilms. *Nat. Rev. Microbiol.* **14**, 251 (2016).

Beer, C. et al. Terrestrial gross carbon dioxide uptake: global distribution and covariation with climate. *Science*, **329**, 834-838 (2010).

Benke, A. C. *et al.* Length–mass relationships for freshwater macroinvertebrates in North America with particular reference to the southeastern United States. *J. N. Am. Benthol. Soc.* **18**, 308–343 (1999).

Berg, B. & Meentemeyer, V. Litter quality in a north European transect versus carbon storage potential. *Plant Soil,* **242**, 83–92 (2002).

Boulton, A.J. The functional role of the hyporheos. *Verh. Internat. Verein. Limnol*. **27**, 51–63 (2000).

Boulton, A.J. & Boon, P.I. A review of methodology used to measure leaf litter decomposition in lotic environments: time to turn over an old leaf?. *Mar. Freshwater Res.* **42**, 1–43 (1991).

Boulton, A.J. et al. The functional significance of the hyporheic zone in streams and rivers. *Annu Rev Ecol Evol Syst.* **29**, 59–81 (1998).

Boyero, L. et al. A global experiment suggests climate warming will not accelerate litter decomposition in streams but might reduce carbon sequestration. *Ecol. Lett.* **14**, 289–294 (2011).

Bratbak, G. & Dundas, I.A.N. Bacterial dry matter content and biomass estimations. *Appl. Environ. Microbiol.* **48**, 755–757 (1984).

Burrows, R.M. *et al.* High rates of organic carbon processing in the hyporheic zone of intermittent streams. Sci. Rep. 7, 13198 (2017).

Cebrian, J. & Duarte, C.M. Plant growth–rate dependence of detrital carbon storage in ecosystems. Science, 268, 1606–1608 (1995).

Chao, A. et al. Rarefaction and extrapolation with Hill numbers: a framework for sampling and estimation in species diversity studies. *Ecol. Monographs*, **84**, 45-67 (2014).

Cole, J. J. et al. Plumbing the global carbon cycle: integrating inland waters into the terrestrial carbon budget. *Ecosystems*, **10**, 172–185 (2007).

Cornut, J. et al. Early stages of leaf decomposition are mediated by aquatic fungi in the hyporheic zone of woodland streams. *Freshwater biol.* **55**, 2541–2556 (2010).

Danger, M. et al. Effects of burial on leaf litter quality, microbial conditioning and palatability to three shredder taxa. *Freshwater biol.* **57**, 1017–1030 (2012).

Datry, T. et al. A global analysis of terrestrial plant litter dynamics in non–perennial waterways. *Nat. Geosci.* **1** (2018).

Djukic, I. et al. Early stage litter decomposition across biomes. *Sci. Total. Environ.* **628**, 1369–1394 (2018).

Dole‐Olivier, M. J. Surface water–groundwater exchanges in three dimensions on a backwater of the Rhône River. *Freshwater biol.* **40**, 93–109 (1998).

Dunscombe, M. et al. Community structure and functioning below the streambed across contrasting geologies. *Sci. Total. Environ.* **630**, 1028–1035 (2018).

Ellis, B. et al. Flowcore: basic structures for ow cytometry data. R package version 1.10.0. (2009) https://www.bioconductor.org/packages/release/bioc/html/flowCore.html

Febria, C. M. et al. Bacterial community dynamics in the hyporheic zone of an intermittent stream. *ISME J.* **6**, 1078 (2012).

Feigl, V. et al. Influence of red mud on soil microbial communities: Application and comprehensive evaluation of the Biolog EcoPlate approach as a tool in soil microbiological studies. *Sci. Total. Environ.* **595**, 903–911 (2017).

Feller, R. J. & Warwik, R. M. in *Introduction to the study of meiofauna* (eds Higgins, R. P. & Thiel, H.) 181—196 (Smithsonian Institution Press, Washington, 1998).

Findlay, S. Importance of surface‐subsurface exchange in stream ecosystems: The hyporheic zone. *Limnol. Oceanogr.* **40**, 159–164 (1995).

Findlay, S. E. & Arsuffi, T. L. Microbial growth and detritus transformations during decomposition of leaf litter in a stream. *Freshwater biol.* **21**, 261–269 (1998).

Fischer, H. et al. Differential retention and utilization of dissolved organic carbon by bacteria in river sediments. *Limnol. Oceanogr.* **47**, 1702–1711 (2002a).

Fischer, H. et al. Bacterial abundance and production in river sediments as related to the biochemical composition of particulate organic matter (POM). *Biogeochemistry*, **61**, 37–55 (2002b).

Foissner, W. & Berger, H. A user‐friendly guide to the ciliates (Protozoa, Ciliophora) commonly used by hydrobiologists as bioindicators in rivers, lakes, and waste waters, with notes on their ecology. *Freshwater. Biol*. **35**, 375–482 (1996).

Foucreau, N. et al. Effects of rising temperature on a functional process: consumption and digestion of leaf litter by a freshwater shredder. *Fund. Appl. Limnol.* **187**, 295–306 (2016).

Foulquier, A. et al. Response of sediment biofilm to increased dissolved organic carbon supply in groundwater artificially recharged with stormwater. *J. Soils Sediments*, **11**, 382–393 (2011).

Fuhrman, J. A. & Azam, F. Bacterioplankton secondary production estimates for coastal waters of British Columbia, Antarctica, and California. *Appl. Environ. Microbiol.* **39**, 1085–1095 (1980).

Foucreau, N. et al. Effects of rising temperature on a functional process: consumption and digestion of leaf litter by a freshwater shredder. *Fund. Appl. Limnol /Archiv Hydrobiol*. **187**, 295-306 (2016).

Gessner, M.O. et al. A perspective on leaf litter breakdown in streams. *Oikos*, **85**, 377–384 (1999).

Gessner, M.O. et al. Diversity meets decomposition. Trends Ecol. Evol. 25, 372–380 (2010).

Gessner, M.O. & Chauvet, E. Importance of stream microfungi in controlling breakdown rates of leaf litter. *Ecology*, **75**, 1807-1817 (1994).

Golladay, S. W. Factors affecting food utilization by a leaf shredding aquatic insect: leaf species and conditioning time. *Ecography*, **6**, 157–162 (1983).

Gonçalves, J. F. et al. Litter decomposition in a Cerrado savannah stream is retarded by leaf toughness, low dissolved nutrients and a low density of shredders. *Freshwater biol.* **52**, 1440–1451 (2007).

Gonçalves, J. F. et al. Factors controlling leaf litter breakdown in Amazonian streams. *Hydrobiologia*, **792**, 195–207 (2017).

Gryta, A. et al. The application of the Biolog EcoPlate approach in ecotoxicological evaluation of dairy sewage sludge. *Appl. Biochem. Biotechnol.* **174**, 1434–1443 (2014).

Gulis, V. & Suberkropp, K. Leaf litter decomposition and microbial activity in nutrient‐enriched and unaltered reaches of a headwater stream. *Freshwater biol.* **48**, 123–134 (2003).

Hsieh, T. C. et al. iNEXT: iNterpolation and EXTrapolation for species diversity. R package version 2.0. (2014). http://CRAN.Rproject.org/package=iNEXT.

Huang, S. et al. Characterization of depth–related bacterial communities and their relationships with the environmental factors in the river sediments. *World J. Microbiol. Biotechnol.* **27**, 2655–2664 (2011).

Jansson M., et al. Impact of allochthonous organic carbon on microbial food web carbon dynamics and structure in Lake Örträsket. *Archiv fur hydrobiologie*, **144**, 409–428 (1999).

Jonsson, M. & Malmqvist, B. Ecosystem process rate increases with animal species richness: evidence from leaf‐eating, aquatic insects. *Oikos*, **89**, 519–523 (2000).

Kenarova, A. et al. Community level physiological profiles of bacterial communities inhabiting uranium mining impacted sites. *Ecotoxicology environ. Safety*, **100**, 226–232 (2014).

Keuskamp, J.A. et al. Tea Bag Index: a novel approach to collect uniform decomposition data across ecosystems. *Methods Ecol. Evol*. **4**, 1070-1075 (2013).

Knapp, J. L. *et al.* Tracer‐based characterization of hyporheic exchange and benthic biolayers in streams. *Water Resour. Res*. **53**, 1575–1594 (2017).

Kristensen, E. et al. What is bioturbation? The need for a precise definition for fauna in aquatic sciences. *Mar. Ecol. Prog. Ser.* **446**, 285–302 (2012).

Kuznetsova, A. et al. lmerTest: Tests in Linear Mixed Effects Models. R Package Version 2.0–20. R Foundation for Statistical Computing, Vienna (2015). https://cran.r–project.org/web/packages/lmerTest/index.html.

Kuznetsova, A. et al. lmerTest package: Tests in linear mixed effects models. *J. Stat. Softw.* **82**, 1–26 (2017).

Maridet, L. & Philippe, M. Influence of substrate characteristics on the vertical distribution of stream macroinvertebrates in the hyporheic zone. *Biologia*, **91**, 101–105 (1995).

Marmonier, P. et al. The role of organisms in hyporheic processes: gaps in current knowledge, needs for future research and applications. *Ann. Limnol.–Int. J. Limnol.* **48**, 253–266 (2012).

McKie, B. G. Ecosystem functioning in stream assemblages from different regions: contrasting responses to variation in detritivore richness, evenness and density. *J. Anim. Ecol.*, **77**, 495–504 (2008).

Naamane, B. et al. The breakdown of leaves of poplar and holm oak in three Moroccan streams: effect of burial in the sediment. *Ann. Limnol.–Int. J. Limnol.* **35**, 263–275 (1999).

Nakagawa, S. & Schielzeth, H. A general and simple method for obtaining R2 from generalized linear mixed‐effects models. *Methods Ecol. Evol.* **4**, 133–142 (2013).

Navel, S. *et al.* Interactions between fauna and sediment characteristics control plant matter breakdown in river sediments. *Freshw. Biol.* **55**, 753–766 (2010).

Navel, S. et al. Water–sediment exchanges control microbial processes associated with leaf litter degradation in the hyporheic zone: a microcosm study. *Microb. Ecol.* **61**, 968–979 (2011).

Pascoal, C. & Cássio, F. Contribution of fungi and bacteria to leaf litter decomposition in a polluted river. *Appl. Environ. Microbiol.* **70**, 5266–5273 (2004).

Peralta–Maraver, I. et al. Macroinvertebrate colonization of two different tree species leaf packs (native vs. introduced) in a Mediterranean stream. *J. Freshw. Ecol.* **26**, 495–505 (2011).

Peralta–Maraver, I. et al. Interplay of hydrology, community ecology and pollutant attenuation in the hyporheic zone. *Sci. Total. Environ.* **610**, 267–275 (2018a).

Peralta–Maraver, I. et al. Environmental filtering and community delineation in an ecotone ecosystem. *Sci. Rep.* **8***,* 15871.

Prescott, C.E. Litter decomposition: what controls it and how can we alter it to sequester more carbon in forest soils? *Biogeochemistry*, **101**, 133–149 (2011).

Putt, M. & Stoecker, D. K. An experimentally determined carbon: volume ratio for marine "oligotrichous" ciliates from estuarine and coastal waters. *Limnol. Oceanogr.* **34**, 1097–1103 (1989).

R Core Team. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria (2018). http://www.R–project.org.

Reiss, J. & Schmid–Araya, J. M. Existing in plenty: abundance, biomass and diversity of ciliates and meiofauna in small streams. *Freshwater biol.* **53**, 652–668 (2008).

Reiss, J. & Schmid–Araya, J. M. Life history allometries and production of small fauna. *Ecology*, **91**, 497–507 (2010).

Reynolds Jr, S. K. & Benke, A. C. Chironomid production along a hyporheic gradient in contrasting stream types. *Freshw. Sci.* **31**, 167–181 (2012).

Ribblett, S. G. et al. The importance of bacterivorous Protozoa in the decomposition of stream leaf litter. *Freshwater biol.* **50**, 516–526 (2005).

Risse–Buhl, U. Aquatic Protozoa modulate the microbial activity associated with mineral surfaces and leaf litter. *Aquat. Microb. Ecol.* **66**, 133–147 (2012).

Robertson, A. L. Lotic meiofaunal community dynamics: colonisation, resilience and persistence in a spatially and temporally heterogeneous environment. *Freshwater biol.* **44**, 135–147 (2000).

Robertson, A. L. et al. Stream hydraulics and the distribution of microcrustacea: a role for refugia?. *Freshwater biol.* **33**, 469–484 (1995).

Robertson, A. L. & Wood, P. J. Ecology of the hyporheic zone: origins, current knowledge and future directions. *Fund. Appl. Limnol.* **176**, 279–289 (2010).

Romaní, A. M. et al. Relevance of polymeric matrix enzymes during biofilm formation. *Microb. Ecol.* **56**, 427–436 (2008).

Rundle, S. D. et al. *Freshwater meiofauna*: Biology and Ecology. (Blackhuys Publishers, Leiden, 2002).

Sabater, S. et al. Effect of climate on the trophic structure of temperate forested streams. A comparison of Mediterranean and Atlantic streams. *Sci. Total. Environ.* **390**, 475–484 (2008).

Sarkar, D. et al. Using flowViz to visualize flow cytometry data. *Bioinformatics,* **24**, 878–879 (2008).

Schaum, C. E. et al. Adaptation of phytoplankton to a decade of experimental warming linked to increased photosynthesis. *Nat. Ecol. Evol.* **1**, 0094 (2017).

Schmid–Araya, J. M. Temporal and spatial distribution of benthic microfauna in sediments of a gravel streambed. *Limnol. Oceanogr.* **39**, 1813–1821 (1994).

Schneider, T. *et al.* Who is who in litter decomposition? Metaproteomics reveals major microbial players and their biogeochemical functions. *ISME J.* **6**, 1749 (2012).

Shapiro O.H. et al. Bacteriophage predation regulates microbial abundance and diversity in a full–scale bioreactor treating industrial wastewater. *ISME J.***4**, 327 (2010).

Singer, G. *et al.* Physical heterogeneity increases biofilm resource use and its molecular diversity in stream mesocosms. *PLoS One*, **5**, e9988 (2010).

Sliva, L. & Williams, D. D. Responses of hyporheic meiofauna to habitat manipulation. *Hydrobiologia*, **548**, 217–232 (2005).

Smith, J. J. & Lake, P. S. The breakdown of buried and surface–placed leaf litter in an upland stream. *Hydrobiologia*, **271**, 141–148 (1993).

Stanley, E. H. et al. Ecosystem expansion and contraction in streams. *BioScience*, **47**, 427–435 (1997).

Stead, T. K. et al. The contribution of subsurface invertebrates to benthic density and biomass in a gravel stream. *Arch. Hydrobiol.* **160**, 171–191 (2004).

Strayer, D. L. et al. Oxygen, organic matter, and sediment granulometry as controls on hyporheic animal communities. *Arch. Hydrobiol.* **140**, 131–144 (1997).

Tachet, H. et al. *Invertébrés d'eau douce. Systématique, biologie, écologie.* (Centre National de la Recherche Scientifique editions, Paris, 2010).

Tank, J. L. *et al.* A review of allochthonous organic matter dynamics and metabolism in streams. *J. N. Am. Benthol. Soc.* **29**, 118–146 (2010).

Thompson, M. S. et al. Large woody debris "rewilding" rapidly restores biodiversity in riverine food webs. *J. Appl. Ecol.* **55**, 895-904 (2017).

Tiegs, S.D. et al. Cotton–strips as a leaf surrogate to measure decomposition in river floodplain habitats. *J. N. Am. Benthol. Soc.* **26**, 70–77 (2007).

Tiegs, S.D. et al. A standardized cotton–strip assay for measuring organic–matter decomposition in streams. *Ecol. Indic.* **32**, 131–139 (2013).

Tod, S. P. & Schmid–Araya, J. M. Meiofauna versus macrofauna: secondary production of invertebrates in a lowland chalk stream. *Limnol. Oceanogr.* **54**, 450–456 (2009).

Townsend, C. R. et al. The intermediate disturbance hypothesis, refugia, and biodiversity in streams. *Limnol. Oceanogr.***42**, 938–949 (1997).

Usio, N. & Townsend, C. R. Roles of crayfish: consequences of predation and bioturbation for stream invertebrates. *Ecology*, **85**, 807-822 (2004).

Vannote, R. L. et al. The river continuum concept. *Can. J. Fish. Aquat. Sci.* **37**, 130–137 (1980).

Watson, S.W. et al. Determination of bacterial number and biomass in the marine enviroment. *Appl. Enviro. Microbiol.* **33**, 940–946 (1977).

Webster, J.R. & Benfield, E.F. Vascular plant breakdown in freshwater ecosystems. *Annu. Rev. Ecol. Evol. Syst.* **17**, 567–594 (1986).

Whigham, D.F. et al. Watershed influences on the structure and function of riparian wetlands associated with headwater streams–Kenai Peninsula, Alaska. *Sci. Total. Environ.* **599**, 124–134 (2017).

Woodward, G. et al. Continental–scale effects of nutrient pollution on stream ecosystem functioning. *Science*, **336**, 1438–1440 (2012).

Yadav, V. & Malanson, G. (2007). Progress in soil organic matter research: litter decomposition, modelling, monitoring and sequestration. *Prog. Phys. Geogr.* **31**, 131–154.

Zuur, A. F. *et al. Mixed Effects Models and Extensions in Ecology with R*. (Springer, New York, 2009).

Chapter 6 | General discussion

While ecologists involved in management or policy often are advised to learn to deal with uncertainty, there are a number of components of global environmental change of which we are certain — certain that they are going on, and certain that they are human-caused. Some of these are largely ecological changes, and all have important ecological consequences.

Peter M. Vitousek (1994)

The over-arching aims of this body of work were to determine how the whole stream bed community changes with depth and hydrologic exchange, examine the relationship of the hyporheos with lotic ecosystem processes and services, and assess the relative importance of these processes depending on the streambed compartment. I explored my research questions in a variety of ways ranging from highly controlled microcosm experimentation miming sediment conditions to a regional field survey of 30 streams. My conclusions are based on robust results generated by large sample sizes/ replication. My approach to this research was two-fold. Firstly, it was iterative whereby the findings of one section were used as a springboard for the research undertaken in the next. Thus the chapters of this thesis are strongly interrelated. Secondly, it was multi-disciplinary and took an integrative strategy, a necessity in the complex environment that is the HZ. A conceptual diagram showing how my research has enlightened our general understanding of processes (and their drivers) in the streambed is available as Fig 6.1. This diagram also shows the scale of study and logical future research steps from my findings.

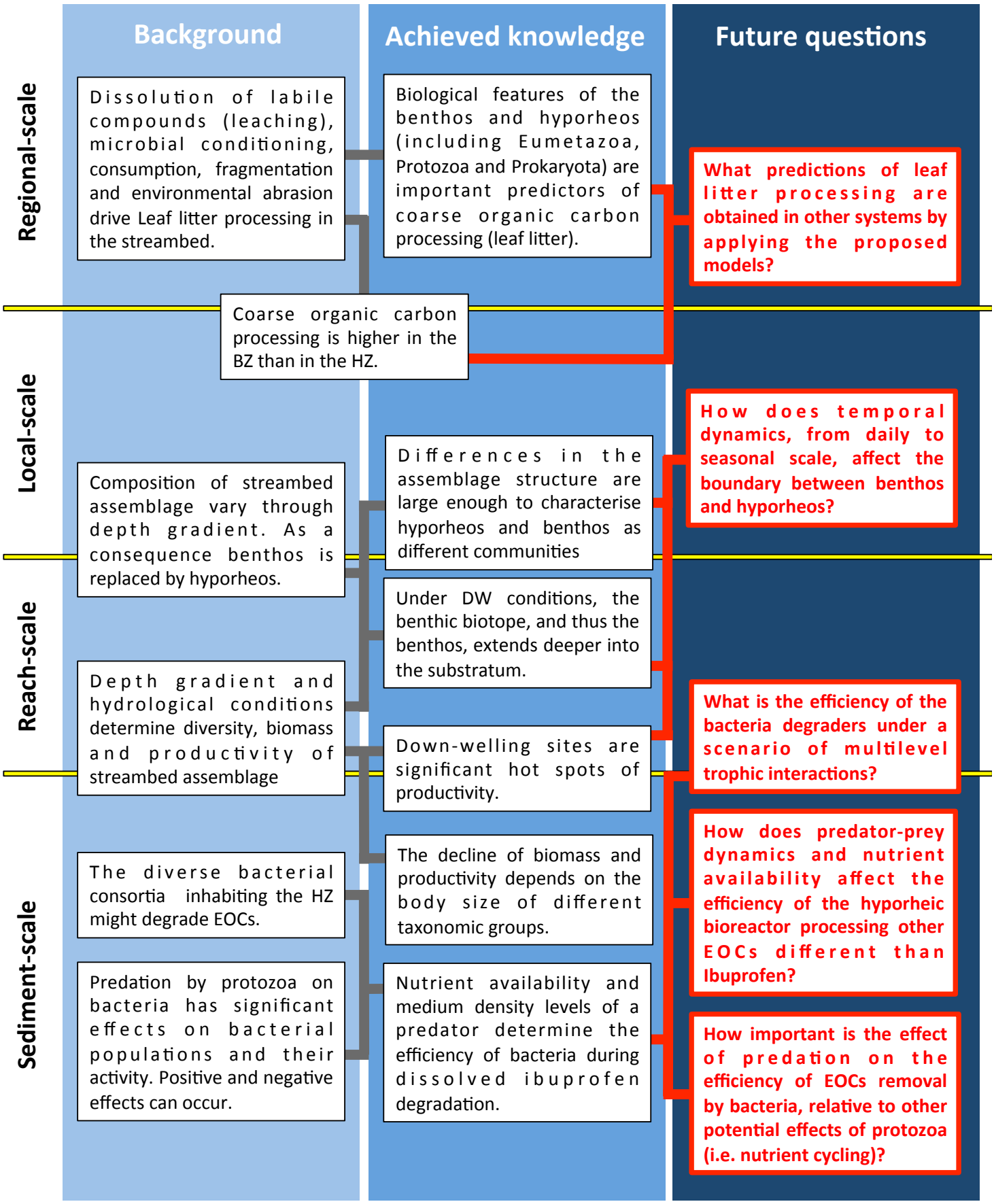

Fig 6.1. Conceptual diagram summarising the main background of this thesis, main achieved knowledge, and future research questions arisen from current findings, and the scale of relevance. Grey lines show how the background is related with the new findings; while red lines show how future questions arise from the achieved knowledge. Intensity of blue in the background is related with the

1 | Highlights

In summary, the main achieved results of my thesis are:

1.1 | Biomass and productivity decrease through the depth gradient depending on the body size of different taxonomic groups.

1.2 | Down-welling (DW) sites are significant hot spots of productivity, and therefore carbon processing in streambed systems.

1.3 | Hyporheos and benthos are measurable ecological communities with individual integrity.

1.4 | Under DW conditions, benthos extends deeper into the substratum than under UW conditions.

1.5 | Nutrient availability and predation by a ciliate species determines how efficient bacteria are at degrading dissolved ibuprofen in microcosms simulating the pore spaces in the HZ.

1.6 | There is a positive synergic interaction between the effect of predators and the increase of degrader cell density, which results in an intensification of the dissolved ibuprofen consumption in microcosms simulating the pore spaces in the HZ.

1.8 | Leaf litter processing is higher in benthic zone (BZ), while the HZ has a more important role in the sequestration and storage of organic carbon.

1.9 | The whole assemblage of organisms (including Prokaryota, Protozoa and Eumetazoa invertebrates) is involved during the leaf litter processing in the streambed.

1.10 | Compartmentalization of the system (BZ and HZ) and the biological features of the assemblage of organisms are robust predictors of the leaf litter processing in the streambed.

2 | The wider context of my research

Modern ecology is certainly a science of urgency. Within the actual framework of global change, understanding the ecological processes may become a race against the clock in which environmental scientists have to deal with uncertainty (Vitousek 1994). A direct consequence is that ecological research is increasingly focused on complex ecosystems processes and services (i.e. Stegen et al. 2016, Mendoza-Lera & Datry 2017) and this often means sacrificing knowledge of the environment where these processes occur or the communities that may play a central role in them (Underwood et al. 2000). Ecologists are often increasingly hesitant to initiate and invest in descriptive field studies. This is also reflected in the reduced spectrum of taxonomic groups involved in the study of many biological communities (Mayr 1997). This is certainly counterproductive because descriptive studies are critical in providing the context and basis for studying mechanisms and processes that occur within natural systems (Underwood et al. 2000). Ecological research on freshwater systems and particularly in the HZ, the focus of this thesis, is also guilty of this approach. It is shocking that despite the large number of studies that have assessed the role of organisms in complex hyporheic processes (Marmonier et al. 2012), since the HZ was first defined by Orghidan (1955), the existence of a measurable transition between benthos and hyporheos had never been delimited quantitatively in the field before (Fig 6.1: Background).

In order to acquire a better knowledge of ecosystems, and by extension a greater understanding of the complex processes of the natural world, modern science is increasingly conducted by collaborative and multidisciplinary teams (Wuchty *et al.* 2007, Oliver *et al.* 2018). Embracing this perspective, I first included a wide range of study groups (micro-, meio- and macrofauna) in my study design, a demanding

undertaking (Reiss & Schmid-Araya 2010, Peralta-Maraver 2018). Then, by coupling methodologies imported from hydrology, biochemical engineering and community ecology I described how environmental filtering (depth and redox gradient, hydrological conditions and streambed compartmentalization) shapes the assemblage in the streambed (Fig 6.1: Achieved knowledge at reach- and local-scale). As discussed in chapter 3, the revealed interaction between taxonomic group (based on body size) and the decline in biomass and productivity shed light on our limited understanding of the net production of streambed systems (Reiss & Schmid-Araya 2010) and improved the reliability of the previous proposed predictive models of this process (Peralta-Maraver *et al.* 2018)(Fig 6.1: Achieved knowledge at sediment-scale). I also characterised benthos and hyporheos as unambiguously discrete communities and located the boundary between them as the biological limit between the BZ and the HZ (Fig 6.1: Achieved knowledge at sediment-scale). Apart from the theoretical connotations of this assessment (discussed in Chapter 3), delimiting streambed system boundaries is the first step to locating and measuring the processes and services that occur within the streambed (Naiman *et al.* 1988, Smock *et al.* 1992, Post *et al.* 2007). This approach also makes it possible to characterise the differential contribution of benthos and hyporheos to these processes and services (as reported in Chapter 5). My findings also draw attention to potential limitations of previous studies in which authors compared the HZ and the BZ in terms of budgets of diversity, biomass and productivity based on operational demarcation of the system (Smock *et al.* 1992, Collier *et al.* 2004, Wright-Stow *et al.* 2006).

Describing the ecological features of the biotope and biocenosis in the BZ and the HZ laid the groundwork for the study of more complex mechanisms behind ecosystem processes. In Chapter 4, I experimentally proved the positive effect of nutrient

availability and predation by Protozoa on the removal of emerging organic contaminants (EOC) by colloidal bacteria inhabiting the HZ (Fig 6.1: Achieved results at sediment-scale). In combination with the reported decline in biomass through depth and under UW conditions in Chapter 3, it might be hypothesised that there is a concomitant reduction on the positive effect of predators on the removal of EOCs. It could also partially explain the reported decline in the biochemical turnover at the lower streambed layers (Battin *et al.* 2003, O'Connor and Harvey 2008, Knaap *et al.* 2017). Results from Chapter 5 reinforce this suggestion. In this chapter I showed that processing of allochthonous coarse organic matter (leaf litter) is significantly lower in the HZ than in the BZ (Fig 6.1: Background and Achieved results at local- and regional-scale). More importantly, my findings highlight that all the components of benthos and hyporheos are essential gears operating directly or indirectly on the functioning of the streambed bioreactor (in this case leaf litter processing). Thus, even accepting the central role of degraders (Prokaryota and fungi, although the latter were not assessed in this body of work) in the process, their efficiency might depend largely on the interaction with the rest of the community.

3 | Future research steps

My thesis addresses significant knowledge gaps that were identified in previous research (Smock et al. 2012, Robertson & Wood 2010, Reiss & Schmid-Araya 2010, Peralta-Maraver *et al.* 2018), such as the role of Protozoa and meiofauna in the budget of biomass, diversity and secondary production of the streambed, the location of the boundary between benthos and hyporheos, and the involvement of the assemblage of organisms during the nutrient and contaminant transformation in the BZ and the HZ. However, there are still interesting unanswered ecological questions (see figure 6.1), in

addition to those that have arisen from my current research, as I explain in the following five paragraphs.

3.1 | I did not assess how the temporal dynamic affects the ecological processes in the BZ and the HZ. As discussed in Chapter 2, the temporal dynamic (from daily- to seasonal-scale) affects the hydrological exchange conditions between the BZ and the HZ (Gibert *et al.* 1990, Kalbus *et al.* 2006) and the organization of the streambed biota (Townsend *et al.* 1997, Robertson 2000). Hence, it is reasonable to expect that the location of the boundary between benthos and hyporheos is time-dependent. Supplementary analysis in Chapter 3 showed that line of demarcation between benthos and hyporheos tended to be relatively persistent over the study period (Table S3.4) although slight variations in the similarity suggest that location of the boundary between communities might vary marginally. However, it was not possible to test the magnitude of these variations due to the lack of temporal replication. On the one hand, future survey studies might assess the integrity of benthos and hyporheos at a daily-scale by increasing the frequency of sampling times. Recalling the daily variation of the surface water level caused by the waste water treatment plant (WWTP) release, a reasonable strategy would be repeating the sampling collection during the maximum and minimum water stage level (Fig 3.2). On the other hand, assessing the seasonal variation in the boundary between both communities implies repeating the same protocols during different seasons. Based on my achieved knowledge (Fig 6.1: Achieved results at sediment-, reach and local-scale), sampling effort would be notably minimized by reducing spatial scale.

3.2 | The apparently simple set up of the microcosms experiment presented in Chapter 4, was preceded by a large effort to define the experimentation protocol and so test my hypothesis. Furthermore, this study was the first to assess the role of predation on

biodegradation of EOCs in a natural bioreactor. However, the reduction in complexity may limit the transfer of my findings to a field situation. After testing the effect of a simple three-levels food chain on Ibuprofen removal, a logical next step might be to assess the effect of multilevel trophic interactions in the process (Fig 6.1: Future questions at sediment scale). Similar experimental protocols (using microcosms experiments) including second level predators and/or increasing richness of basal resources might test more realistic situations and results might be compared with those achieved in my actual experiment.

3.3 | Related with the above, complementary experiments should also consider different EOCs in order to draw general conclusions (Fig 6.1: Future questions at sediment scale). In my microcosms experiment, Ibuprofen was used as a carbon source by the bacteria, but EOCs include a large variety of molecular compounds (Pal *et al.* 2010) and therefore, the way in which bacteria could use them and their efficiency in removing them from the water must be equally variable. Experiments testing the hyporheic bioreactor efficiency to process different families of EOCs will characterize the level of risk of these compounds for the environment.

3.4 | Likewise, a promising following step might be to test the effect of predation on the efficiency of EOCs removal by bacteria, relative to other potential effects of protozoa (i.e. recycling of nutrients). As reported by Tso & Taghon (2006), this might be possible by using cytochalasin B (a fungal metabolite that inhibits food vacuole formation in protozoa) with the heterotrophs *Tetrahymena pyriformis*. Initial control experiments would be conducted to determine whether cytochalasin B affects bacterial consumption of EOC and to confirm its inhibitory effect on feeding of *T. pyriformis*. Then, the effects of reduced predation could be tested comparing treatments with cytochalasin B and treatments without it (a new factor with 2 levels in my previous experimental design).

3.5 | Finally, my predictive models of leaf litter processing (Chapter 5) were very robust. Thus, applying these coefficients to existing data on diversity and biomass of benthos and hyporheos should make it possible to locate hot spots of alochtonous coarse organic carbon turnover in other systems, and also predict the rate of processing. Analysing metadata from different regions by applying my models might help to draw a broad picture of the distribution of hot spot of carbon processing at a global scale.

4 | Limitations, shortcomings and potential methodological problems

Generally, the way in which ecology researchers approximate natural phenomena (sampling) raises many methodological problems. Specifically, results from Chapter 5 ("Predicting leaf litter decay in the streambed: response to system compartmentalization and involvement of the whole assemblage of organisms") have to be taken with caution regarding the methodology applied to determine leaf litter breakdown. As discussed, the use of standardized bioassays (cotton–strips and tea–bags) was a strategy to solve problems related with traditional leaf pack use in large–scale studies (i.e. species–specific chemical composition of leaves, differences in leaves size and palatability). However, even though these methods have been largely used in published works (see Chapter 5), we cannot ignore a great limitation that this methods brings: cotton–strips and tea–bags are very artificial carbon substrata for streambed communities and therefore, organisms may not be fully adapted to their use. In this vein, the obtained and discussed patterns might be artefacts to a certain degree. Still, our great sampling effort at a regional scale (30 different rivers in UK) strengthens the validity of our findings.

5 | Final conclusions

My thesis has contributed to an increased understanding of streambed characteristics and functionality, with a special emphasis on the HZ and its interaction with the wider stream ecosystem. To a large extent, the knowledge goals achieved here opened a new line of multidisciplinary field studies and experimental protocols that need to be developed during future years. Some of my methods, such as my approach delimitating natural communities along ecotones or my experimental protocol testing the effect of predation on bioreactors functioning, can be extrapolated and tested in different ecosystems such as forests or marine environments. Moreover, both the methodologies and the predictive models developed during this thesis are of interest not only in theoretical ecology, but also in the management and preservation of important ecosystem services in streams and rivers.

6 | References

Battin, T.J. *et al.* Contributions of microbial biofilms to ecosystem processes in stream mesocosms. *Nature*. **426**, 439–442 (2003).

Collier, K.J. *et al.* Trophic basis of production for a mayfly in a North Island, New Zealand, forest stream: contributions of benthic versus hyporheic habitats and implications for restoration. *N. Z. J. Mar. Freshwater Res.* **38**, 301–314 (2004).

Edwards, M.A. & Roy, S. Academic research in the 21st century: Maintaining scientific integrity in a climate of perverse incentives and hypercompetition. *Environ. Eng. Sci.* **34**, 51-61 (2017).

Gibert, J. *et al.* in *The Ecology and Management of Aquatic–Terrestrial Ecotones* (eds Naiman, R. J. & Décamps, H) 199–225 (UNESCO, Paris, 1990).

Hughes, B.B. *et al.* Long-term studies contribute disproportionately to ecology and policy. *Bioscience* **67**, 271-281 (2017).

Kalbus, E. *et al.* Measuring methods for groundwater–surface water interactions: a review. *Hydrol. Earth. Syst. Sc.***10**, 873–887 (2006).

Knapp, J.L. *et al.* Tracer-based characterization of hyporheic exchange and benthic biolayers in streams. *Water Resour. Res*. **53**, 1575–1594 (2017).

Marmonier, P. *et al.* The role of organisms in hyporheic processes: gaps in current knowledge, needs for future research and applications. *Ann. Limnol.- Int. J. Lim.* **48**, 253-266 (2012).

Mayr, E. *This is Biology: the Science of the Living World*. (Harvard University Press: Cambridge, MA, USA, 1997).

Meghdadi, A. & Javar, N. Evaluation of nitrate sources and the percent contribution of bacterial denitrification in hyporheic zone using isotope fractionation technique and multi-linear regression analysis. *J. Environ. Managet*. **222**, 54-65 (2018).

Mendoza-Lera, C., & Datry, T. Relating hydraulic conductivity and hyporheic zone biogeochemical processing to conserve and restore river ecosystem services. *Sci. Total Environ*. **579**, 1815-1821 (2017).

Naiman, R.J. *et al.* The potential importance of boundaries of fluvial ecosystems. *J. N. Amber. Benthol. Soc.* **7**, 289-306 (1988).

O'Connor, B.L. & Harvey, J.W. (2008), Scaling hyporheic exchange and its influence on biogeochemical reactions in aquatic ecosystems. *Water Resour. Res*. **44**, W12423.

Oliver, S. K. *et al.* Strategies for effective collaborative manuscript development in interdisciplinary science teams. *Ecosphere*, **9**, e02206 (2018).

Orghidan, T., Un nou domeniu de viata acvatica subterana:'biotopul hiporeic'. *Bull. Sti. sect. Biologie si sti. Agronau. si sect. Geologie si Geogr*. **7**: 657–676 (1955).

Pal, A. *et al.* Impacts of emerging organic contaminants on freshwater resources: review of recent occurrences, sources, fate and effects. *Sci. Total Environ*. **408**, 6062–6069 (2010).

Peralta-Maraver, I. *et al.* Interplay of hydrology, community ecology and pollutant attenuation in the hyporheic zone. *Sci. Total Environ*. **610**, 267-275 (2018).

Post, D.M. *et al.* The problem of boundaries in defining ecosystems: a potential landmine for uniting geomorphology and ecology. *Geomorphology* **89**, 111-126 (2007).

Reiss, J. & Schmid-Araya, J.M. Existing in plenty: abundance, biomass and diversity of ciliates and meiofauna in small streams. *Freshwater. Biol*. **53**, 652-668 (2008).

Reiss, J. & Schmid-Araya, J.M. Life history allometries and production of small fauna. *Ecology* **91**, 497-507 (2010).

Robertson, A.L. Lotic meiofaunal community dynamics: colonisation, resilience and persistence in a spatially and temporally heterogeneous environment. *Freshwater Biol*. **44**, 135–147 (2000).

Smock, L.A. *et al.* macroinvertebrate production in three dimensions: channel surface, hyporheic, and floodplain environments. *Ecology*, **73**, 876–886 (1992).

Stegen, J. C *et al*. Groundwater–surface water mixing shifts ecological assembly processes and stimulates organic carbon turnover. *Nat. Commun. 7*, 11237 (2016).

Townsend, C.R. *et al.* The intermediate disturbance hypothesis, refugia, and biodiversity in streams. *Limnol. Oceanogr*. **42**, 938–949 (1997).

Tso, S.F. & Taghon, G.L. Protozoan grazing increases mineralization of naphthalene in marine sediment. *Microb. Ecolo.* **51**, 460-469 (2006).

Underwood, A.J. *et al.* Observations in ecology: you can't make progress on processes without understanding the patterns. *J. Exp. Mar. Biol. Ecol.* **250**, 97-115 (2000).

Vitousek, P. M. Beyond global warming: ecology and global change. *Ecology*, **75**, 1861-1876 (1994).

Wright-Stow, A.E. *et al.* Hyporheic production is substantially greater than benthic production for a common New Zealand caddisfly. *Hydrobiologia*, **560**, 295-310 (2006).

Wuchty, S. *et al.* The increasing dominance of teams in production of knowledge. *Science*, **316**, 1036-1039 (2007).